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ORIGINAL

MINUTES
OF THE
PHASE I WORKING GROUP
AND THE
BIOCHEMICAL MODULATORS ADVISORY GROUP

November 17-18, 1986

COMPLETED

Investigational Drug Branch
Cancer Therapy Evaluation Program
National Cancer Institute
Bethesda, Maryland

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This document contains the Minutes of U.S. Government meetings: the Phase I Working Group Meeting and the Biochemical Modulators Advisory Group Meeting. If quoted, these Minutes must be identified as such. These Minutes may contain previously unpublished data. Data are preliminary and may be incomplete.

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AGENDA

BIOCHEMICAL MODULATORS ADVISORY GROUP AND PHASE I WORKING GROUP MEETINGS

November 17-18, 1986

November 17--Morning Session

NIH, Building 1, Wilson Hall

BIOCHEMICAL MODULATORS ADVISORY GROUP MEETING

Approaches to Antineoplastic Drug Discovery in the Pharmaceutical Industry and the National Cancer Institute

8:30 a.m.	Introduction and Welcome	Dr. R. Johnson SmithKline Beckman
8:45 a.m.	Drug Discovery Through Cellular Spectrum-Based Screens	Dr. A. Casazza Dr. D. Stringfellow Bristol-Myers Company
9:15 a.m.	Growth Factors as Targets for the Discovery of Novel Drugs	Dr. M. Brattain Bristol-Baylor Laboratory Baylor Univ.
9:45 a.m.	Approaches to the Discovery of Novel Anti-cancer Drugs Using Murine Solid Tumors	Dr. G. Grindey Eli Lilly and Company
10:40 a.m.	COFFEE BREAK	
11:00 a.m.	Mechanism-Based Discovery of Unique Antineoplastic Agents	Dr. C. Mirabelli SmithKline & French Laboratories
11:30 a.m.	Toward the Discovery of Effective, Selective, and Disease-Specific Treatment for Colorectal Adenocarcinoma	Dr. R. Greig SmithKline & French Laboratories
12:00 p.m.	LUNCH	

November 17--Afternoon Session

1:00 p.m.	Discovery and Evaluation of Antineoplastic Agents	Dr. P. McGovren The Upjohn Company
1:20 p.m.	Source of Novel Drugs for the Treatment of Cancer	Dr. P. Aristoff The Upjohn Company
1:40 p.m.	Evaluating Tumor Cell Uniqueness for Drug Therapy	Dr. W. Wierenga The Upjohn Company

BIOCHEMICAL MODULATORS ADVISORY GROUP MEETING (Continued)

**Approaches to Antineoplastic Drug Discovery in
the Pharmaceutical Industry and the
National Cancer Institute (Continued)**

November 17--Afternoon Session (Continued)

- | | | |
|----------------------|--|---|
| 2:00 p.m. | Biological and Biochemical Strategies for
Developing New Antimetabolites and New
Intercalators | Dr. R. Jackson
Dr. R. Leopold, III
Warner-Lambert/Parke-Davis |
| 2:30 p.m. | Implications of Intrinsic and Acquired
Multiple Drug Resistance in Design of New
Agents for the Treatment of Colon Carcinoma | Dr. W. Klohs
Warner-Lambert/Parke-Davis |
| 3:00 p.m. | COFFEE BREAK | |
| 3:20 p.m. | Disease-Oriented Antitumor Drug Screening | Dr. R. Shoemaker
NCI |
| 3:40 p.m. | New Anticancer Drug Discovery Program
at the National Cancer Institute | |
| | I. Synthetic Agents | Dr. V. Narayanan
NCI |
| | II. Natural Products | Dr. M. Suffness
NCI |
| 4:20 p.m. | Implications for the Clinical Development
of Investigational New Anticancer Agents | Dr. D. Hoth
NCI |
| 4:40 p.m. | Discussion | |
| 6:30 to
8:30 p.m. | Reception at the Bethesda Marriott. Please check hotel reader
board for location. | |

PHASE I WORKING GROUP MEETING

Amonafide

8:15 a.m.	Introduction	Dr. G. Sarosy NCI
8:20 a.m.	IV Bolus Q 21 Days	Dr. G. Weiss San Antonio
8:30 a.m.	IV Bolus Q 21 Days IV Q D X 5 Pharmacokinetics	Dr. J. Leiby Dr. L. Malspeis Ohio State Univ.
8:50 a.m.	IV Q D X 5	Dr. S. Legha M.D. Anderson
8:55 a.m.	24-Hour CIV Pharmacokinetics	Dr. R. Richardson Mayo Clinic
9:10 a.m.	Discussion	

FAA

9:25 a.m.	Introduction	Dr. J. Grem NCI
9:30 a.m.	Q 3 Weeks: Clinical	Dr. M. Grever Ohio State Univ.
9:40 a.m.	Q 3 Weeks: Pharmacokinetics	Dr. L. Malspeis Ohio State Univ.
9:50 a.m.	Q 3 Weeks: Clinical	Ms. T. Melink UMCC
10:00 a.m.	Weekly: Clinical	Dr. R. Weiss NCI-Walter Reed
10:10 a.m.	Weekly: Pharmacokinetics	Dr. J. Collins NCI-Walter Reed
10:20 a.m.	Weekly: Clinical	Dr. J. Craig San Antonio
10:30 a.m.	Discussion	
10:40 a.m.	COFFEE BREAK	

November 18--Morning Session (Continued)

PHASE I WORKING GROUP MEETING (Continued)

Trimetrexate

10:55 a.m.	Introduction	Dr. J. Grem NCI
11:00 a.m.	Pediatric Trials: Clinical	Dr. F. Balis NCI
11:10 a.m.	Pediatric Trials: Clinical and Pharmacokinetics	Dr. B. Kamen POG
11:25 a.m.	Discussion	

Merbarone

11:30 a.m.	Introduction	Dr. H. Chun NCI
11:35 a.m.	D X 5 CI	Dr. E. Kraut Dr. L. Malspeis Ohio State Univ.
11:45 a.m.	D X 5 CI	Dr. R. Warrell Dr. C. Young MSKCC
11:55 a.m.	Discussion	

IV 6-Thioguanine

12:00 p.m.	Introduction	Dr. M. Christian NCI
12:05 p.m.	D X 5 CI IV Q D X 5	Dr. J. Rubin Mayo Clinic
12:15 p.m.	Discussion	
12:20 p.m.	LUNCH	

November 18--Afternoon Session

HMBA

1:20 p.m.	Introduction	Dr. H. Chun NCI
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November 18--Afternoon Session (Continued)

PHASE I WORKING GROUP MEETING (Continued)

HMBA (Continued)

1:25 p.m. D X 10 CI

Dr. M. Fanucchi
MSKCC

1:35 p.m. D X 10 CI

Dr. D. Ettinger
Dr. E. Rowinsky
Johns Hopkins

1:45 p.m. Discussion

High-Dose CBDCA

1:50 p.m. Introduction

Dr. H. Christian
NCI

1:55 p.m. Phase I Trial of HD-CBDCA With and
Without Autologous Marrow Rescue

Dr. T. Shea
Dana-Farber

2:05 p.m. Discussion

EORTC

2:10 p.m. Introduction

Dr. H. Pinedo
EORTC

2:15 p.m. 5-AZA-2-Deoxycytidine in
Acute Leukemia

Dr. P. Dodion
Jules Bordet

2:40 p.m. Phase I Trials of DUP 785

Dr. H. Pinedo
EORTC

3:05 p.m. Discussion

The next BMAG/Phase I Meetings will held on March 10-11, 1987, in Building 31C,
Conference Room 10, NIH Campus.

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BIOCHEMICAL MODULATORS ADVISORY GROUP

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PROGRAMS AT BRISTOL-MYERS FOR THE DISCOVERY OF NEW CANCER CHEMOTHERAPEUTIC AGENTS

1) FERMENTATION

BMRIT



WFD



**OTHER B-M FACILITIES
(Collab. with NCI)**

DISCOVERY

DEVELOPMENT I

DEVELOPMENT II

2) ANALOGS

3) TARGETING In collaboration with Oncogen

4) GROWTH FACTORS

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Experimental Models Employed for the Discovery for New Antitumor Drugs

Biochemical systems

Microbial systems

In vitro tumor cells or cell lines

In vivo experimental tumors:

transplanted animals tumors

ascitic

solid

human tumor xenografts

sc

src

ip

Problems Posed by Screening Fermentation Materials for Antitumor Activity

- Past experience based mainly on selection using in vivo ascitic tumors (S 180, L1210, P388)**
- Need to move to new models to achieve:**
 - ability to identify new chemotypes**
 - activity against chemoresistant tumors**
 - maintaining efficiency and sensitivity**

The Ten Leading Cancers Worldwide, In Rank Order of Estimated Annual Occurrence

<u>Rank</u>	<u>Both Sexes Combined</u>	<u>Approximate Number of New Cases Each Year (Males/Females)</u>	<u>Percent</u>
1	Stomach	805,000	12%
2	Lung	671,000	10
3	Breast	604,000	9
4	Colon/rectum	604,000	9
5	Cervix	537,000	8
6	Mouth/pharynx	403,000	6
7	Esophagus	336,000	5
8	Liver	269,000	4
9	Lymph system	269,000	4
10	Prostate	201,000	3
	Subtotal	4,699,000	70
	All Others	2,013,000	30
	Total	6,712,000	100%

Source: Estimates of the Worldwide Frequency of Twelve
Major Cancers: World Health Organization(1984);
WHO International

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Potential Advantages of In Vitro Cell Lines Screening

- 1) Sensitivity to small amounts of natural
or synthetic compounds**
- 2) Rapidity of testing**
- 3) Possibility to get results on a large number
of mouse or human tumors:
selectivity
mechanism of action**

IC-50 (μ G/ML) of Standard Antitumor Agents

	AKR	B16-F10	HTB-31	KB	MOSER	RCA
Adriamycin	4	0.1	0.02	0.02	16	0.02
Bleomycin	250	250	40	250	250	50
Platinol	16	12	4	5	14	7
Mitomycin C	6	5	4	4	8	1
Vincristine	90	90	0.02	0.6	0.3	0.2
VP-16	35	20	12	20	35	5

(from J. Catino et al, 1985)

Percentage of Fermentation Broths with Bioactivity at Various Dilutions

Dilution	AKR	B16-F10	HTB-31	KB	MOSER	RCA
1/4	80.7	87.7	76.5	86.9	69.0	88.8
1/16	44.8	50.7	47.8	52.2	34.1	42.2
1/64	24.1	26.5	28.8	20.4	14.9	17.1
1/256	11.9	12.8	14.3	7.7	7.0	8.6
1/1,024	4.2	6.2	7.9	3.8	3.5	4.1
1/4,096	1.8	3.4	5.4	2.4	2.0	2.2
1/16,384	1.2	2.4	3.6	1.8	1.3	1.5
1/65,536	0.6	1.8	2.4	1.4	0.9	1.1

(from J. Catino et al, 1965)

Predictive Rates for In Vivo P388 Activity

Test System	# In Vitro Actives	# Tested In Vivo	# Active In Vivo	% True Positive ^A
Antimicrobial	162	63	11	17.5
AKR	82	14	6	42.9
B16-F10	122	22	9	40.9
HTB-31	154	14	5	35.7
KB	75	10	5	50.0
MOSER	68	6	3	50.0
RCA	80	11	4	36.4
≥ 2 Cell Lines	116	20	8	40.0
≥ 3 Cell Lines	68	12	7	58.3
≥ 4 Cell Lines	51	10	6	60.0
≥ 5 Cell Lines	36	5	2	40.0

^A % true positive = # active in vivo / # prescreen positive tested in vivo X 100 (from J. Catino et al, 1985)

METHODS FOR SCREENING FERMENTATION BROTHS FOR NEW CYTOTOXIC AGENTS AT BRISTOL-MYERS

BMRIT

Cell cultures: B16, Moser/RCA

+ Antimicrobial test



In vivo test: P388 lp



WFD

Cell cultures: HCT-116, B16, Moser

+ Antimicrobial test



In vivo test: P388 lp



B16 lp

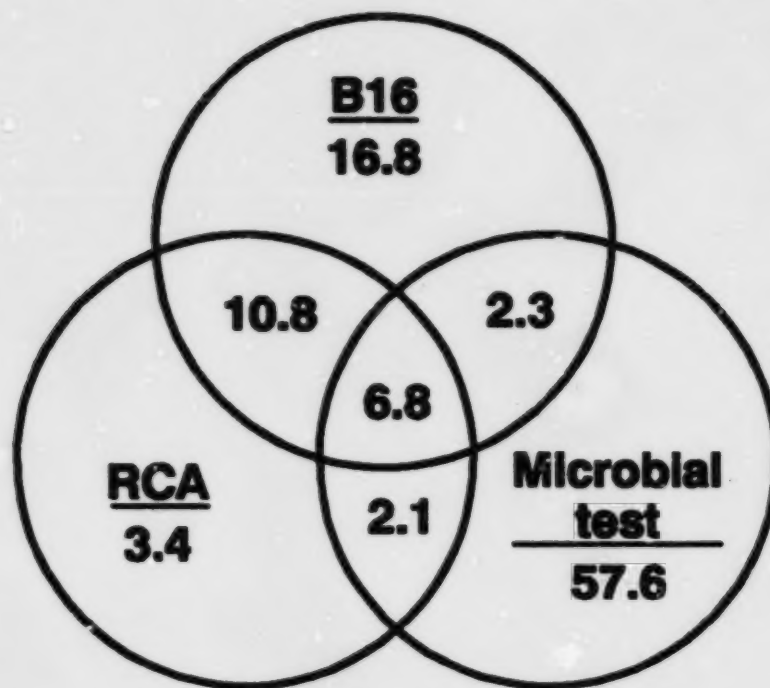
B16 sc

November 1986

Total No. Cultures Tested	No. (%) of Active Cultures	
	B16	RCA
10,192	295 (2.8%)	144 (1.4%)
14,279	442 (3.1%)	199 (1.4%)
24,471	737 (3%)	343 (1.4%)

Data from Dr. Oki and Co. (BMRIT)

% OF CULTURES ACTIVE IN VITRO ON THE DIFFERENT TESTS



CORRELATION BETWEEN IN VITRO AND IN VIVO ACTIVITY

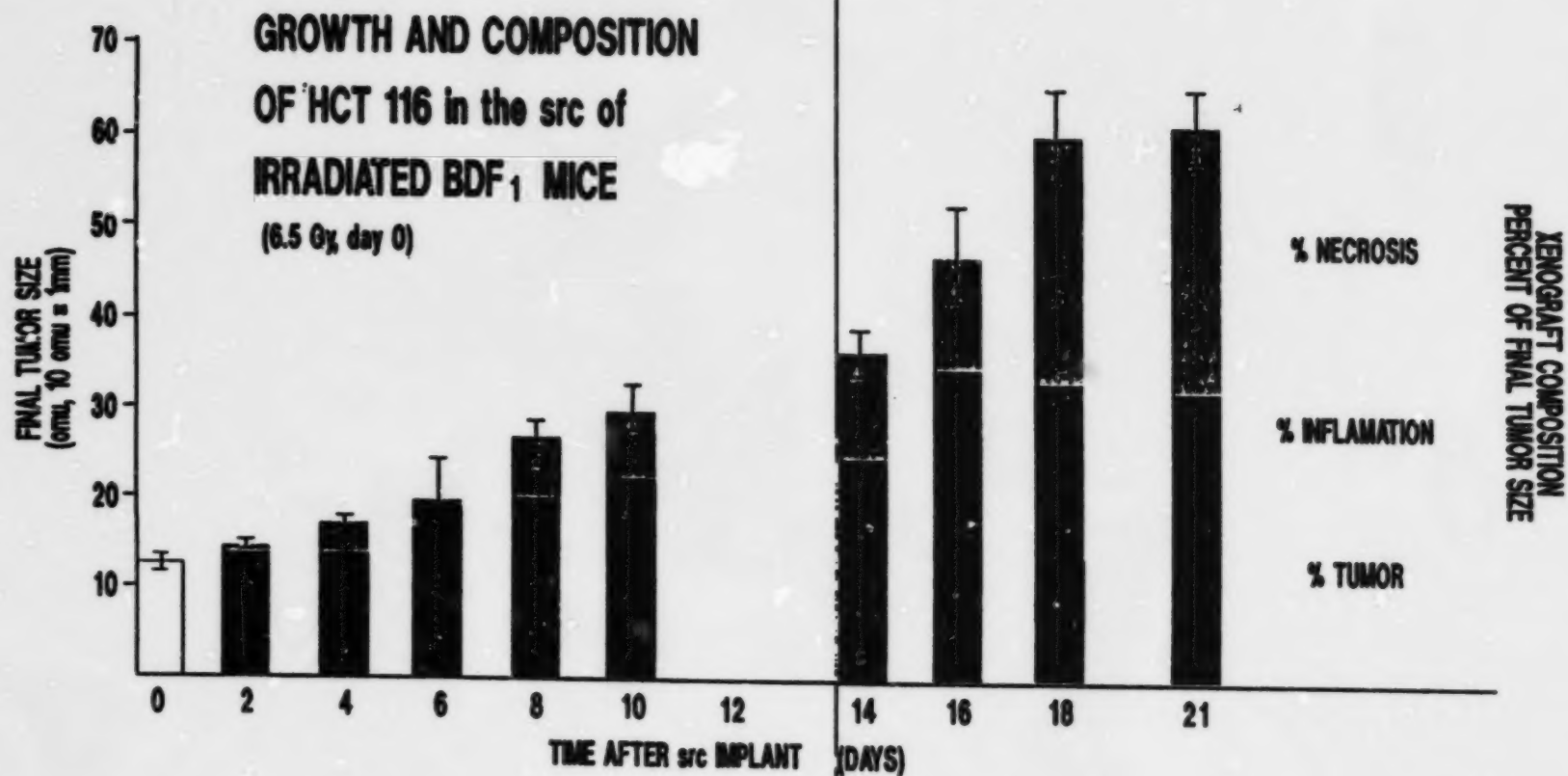
ACTIVITY <u>IN VITRO</u> B16 RCA		No. Actives (%) ON P388 <u>IN VIVO</u>
+		52/198 (26%)
+	+	47/149 (31%)
	+	4/39 (10%)

DATA OBTAINED AT BMRIT (FROM DR. OKI AND Co.)

IN VITRO CELL SYSTEMS (HTSCA, CELL LINES) CAN
IDENTIFY COMPOUNDS WHICH COULD BE MISSED
BY THE USE OF THE CLASSICAL P388 MODEL

PROBLEMS RELATED TO LACK OF ACTIVITY ON P388
IN VIVO:

- A) FORMULATION
- B) RAPID METABOLIC INACTIVATION
- C) SPECIFICITY



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THE EFFECT OF DRUGS ON THE GROWTH OF
HCT 116 IN VITRO AND IN VIVO

DRUG	IN VITRO ACTIVITY	IN VIVO ACTIVITY	
	IC ₅₀ (μG/ML) ^A	% INHIBITION	OPTIMAL DOSE ^B
DOXORUBICIN	0.15	77*	6.0
MITOMYCIN C	0.70	58*	2.4
PLATINOL	7.30	40*	3.0
VP-16	11.6	29	20
IPROPLATIN	25.8	32	10
CARBOPLATIN	58.8	44*	60

^A, IC₅₀, 50% INHIBITORY CONCENTRATION

^B, DOSES WERE ADMINISTERED IN MG/KG/INJ, IV, ON DAYS 1,5, AND 9

*, SIGNIFICANT DIFFERENCE FROM VEHICLE-TREATED CONTROL GROUP
BY STUDENT'S T-TEST (P<0.05)

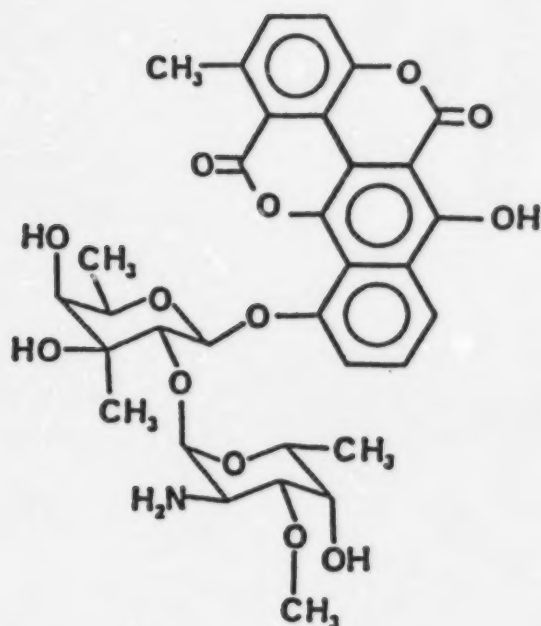
EFFECT OF FERMENTATION BROTH SUPERNATANTS ON TUMOR GROWTH

BROTH	HCT 116	IN VIVO ACTIVITY	
	IN VITRO POTENCY IC ₅₀ (1:)	% INHIBITION OF SRC HCT 116	MAX % T/C vs. IP P388
A-1	4	41	100
A-2	256	41	110
B-1	"	7	156
D-1	"	27	110
A-3	1024	92*	125
D-2	"	74*	100
D-3	4096	47*	111
D-4	"	98*	167
A-4	16384	61*	140
C-1	"	0	111
E-1	"	29	118
A-5	65536	70*	100
A-6	"	85*	120
A-7	"	50*	100
A-8	"	98*	119
A-9	"	41*	130
A-10	"	54*	140
A-11	"	54*	150
B-2	"	77*	139
B-3	"	70*	133

*, SIGNIFICANT DIFFERENCE FROM VEHICLE-TREATED CONTROL BY STUDENT'S T-TEST (P<0.05)

ELSAMICIN

STRUCTURE



-SYNONYMS: BMY-28090
BBM-2478A
ELSAMICIN A
NSC 369327

-PRODUCING STRAIN - MESOPHILIC ACTINOMYCETE FROM EL SALVADOR

-PRODUCTIVITY - 250 MCG/ML

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IN VITRO CYTOTOXICITY OF ELSAMICIN
AND CHARTREUSIN TESTED CONCOMITANTLY

CELL LINE	IC50 (MCG/ML)	
	ELSAMICIN	CHARTREUSIN
B16-F10 (MURINE MELANOMA)	0.14	1.6
HCT-116 (HUMAN COLON)	0.4	5.4
KB (HUMAN NASOPHAR.)	0.52	7
MOSER (HUMAN COLON)	6.5	23.8
M109 (MURINE LUNG)	1.5	17
RCA (HUMAN COLON)	1.1	14

(from J. Schurig, et al; 1986)

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ANTITUMOR ACTIVITY OF ELSAMICIN AGAINST
IP IMPLANTED MURINE TUMORS

TUMOR	TEST LABORATORY	TREATMENT SCHEDULE	OPTIMAL DOSE (MG/KG/INJ, IP)	MST % T/C (CURES/TOTAL)
P388	BRISTOL-MYERS	QD 1-9	4	233
	BRISTOL-MYERS	Q4DX3,D.1	12	289
L1210	NCI	QD 1-5	5	164,177
B16	BRISTOL-MYERS	QD 1-9	6-8	226-261 (0-3/10)
M5076	BRISTOL-MYERS	Q4DX4,D.1	8	>216
	NCI	Q4DX4,D.1	5	146

(from J. Schurig, et al, 1986)

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ANTITUMOR ACTIVITY OF ELSAMICIN AGAINST
SC AND SUBRENAL CAPSULE IMPLANTED MURINE TUMORS

TUMOR	IMPLANT SITE	TREATMENT SCHEDULE, ROUTE	OPTIMAL DOSE (MG/KG/INJ)	% T/C	
				MST	MTW
B16	SC	QD 1-9, IP	A) 6	171	18
			B) 6	130	37
B16	SC	Q4DX3, D.1, IV	A) 16	197	17
			B) 20	170	11
B16	SC	QD 1-5, IV	10	165	25
B16	SRC	Q2DX3, D.1, IV	A) 12	--	30
			B) 10	--	35
M5076	SC	Q4DX4, D.1, IV	16	110	19
M5076	SRC	Q2DX3, D.1, IV	A) 12	--	24
			B) 14	--	0

(from J. Schurig, et al, 1986)

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ACTIVITY OF ELSAMICIN AGAINST SUBRENAL CAPSULE IMPLANTED HUMAN TUMOR XENOGRAFTS

TUMOR	TEST LABORATORY	TREATMENT SCHEDULE, ROUTE	OPTIMAL DOSE (MG/KG/INJ)	MTW % T/C
MX-1 (BREAST)	NCI	Q4DX3,D.1,IP	A) 10	6
			B) 5	76
			C) 10	9
HCT-116 (COLON)	BRISTOL-MYERS	Q4DX3,D.1,IV	A) 12	32
			B) 14	39

(from J. Schurig, et al, 1986)

ANTITUMOR ACTIVITY OF ELSAMICIN AND CHARTREUSIN
TESTED CONCOMITANTLY

TUMOR, SITE	EXP. No.	TREATMENT AND SCHEDULE	OD (MG/KG/INJ)		MST % T/C (MTW % T/C) (CURES/TOTAL)	
			ELSAMICIN	CHARTREUSIN	ELSAMICIN	CHARTREUSIN
P388, IP	7408	QD 1-9, IP	4	64	>335(3/6)	153
B16, IP	601	QD 1-9, IP	6	96	>261	165
B16, SC	601	QD 1-9, IP	6	96	130(37)	105(66)
		Q4DX3, D.1, IV	20	ND	170(11)	—
C26, IP	63	QD 1-4, IP	2	64	116	96
C26, SC	63	QD 1-4, IP	2	64	101(207)	111(156)

(from J. Schurig, et al, 1986)

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CELL LINES PANEL

FERMENTATION:

CELL LINES SELECTED ON THE BASIS OF:
SENSITIVITY TO KNOWN ANTITUMOR AGENTS.

ANALOGS OF ESTABLISHED ANTITUMOR AGENTS:

CELL LINES SELECTED ON BASIS OF:

A) NATURAL

B) INDUCED RESISTANCE TO THE PARENT DRUG
WITH THE OBJECTIVE TO IDENTIFY ANALOGS
SHOWING SELECTIVE CYTOTOXICITY AGAINST
RESISTANT CELL LINES.

ANALOGS EVALUATION NETWORK

CELL LINES	VP-16	ANALOGS TO BE TESTED MMC	CIS-PT
MOUSE MELANOMA B16	S	S	S
HUMAN LUNG A549 SW 900 SW 1271	S R	S R	R S R
HUMAN COLON HCT-116 CBS MOSER RCA	S R R	S R	R R S
HUMAN BREAST MCF-7	R		
INDUCED RESISTANCE B16/MM HCT-116/VP A549/VP A549/MM HCT-116/MMC ? /CIS-PT P388/MMC	R R R	R R R	R (R)?

R = RESISTANT (PARENT DRUG)
S = SENSITIVE (PARENT DRUG)

(CELL LINES SELECTED BY M. BRATTAIN AND B. LONG, BRISTOL-BAYLOR)

IC 50 VALUES FOR VP-16

	IC 50 (μ G/ML)	IC 50 RATIO REL TO HCT-116
HCT-116	4.4-25	1
B16	0.8-18	0.4-1.2
MOSER	38-226	4.1-11.8
CBS	14	3.1
SW1271	2.2	0.3-0.5
M109	53-83	2.2-5.4
HCT-116/VP	110- > 347	5.4- > 14

In Vitro Cell Panel Screening

VP-16 Resistant Cell Line	"Active" Analog[*]	Analog^s Equiv. to VP-16^{**}
Moser	19/21	1/21
HCT-116/VP	4/7	1/7

*** IC50 Ratio < 4 when VP-16 is > 4**

**** IC50 Ratio > 4 when VP-16 is > 4**

ANTITUMOR ACTIVITY OF FERMENTATION
COMPOUND BU SYR
(DATA OBTAINED AT BMRIT, BM, NCI)

TUMOR TYPE	ROUTE OF TUMOR TRANSPLANT	TREATMENT	ACTIVITY
MOUSE TUMORS			
P388	IP	IP	+
	IV	IP, IV	-
P388/MMC	IP	IP	-
L1210	IP	IP	-
B16	IP	IP	-
	SC	IV	-
	SRC	IV	-
M5076	IP	IP	-
M109	IP	IP	+
	SC	IP, IV	-
HUMAN XENOGRAFTS			
HCT-116	SRC	IP, IV	-
MOSER	SRC	IP, IV	-
MX-1	SRC	IP	-
LOX	IP	IP	+

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IC50 VALUES FOR BU-SYR

CELL LINE	IC50(MGM/ML)	IC50 RATIO REL TO HCT116
HCT-116	0.025	1
A549	0.18	7.2
B16-F10	0.12	4.6
MOSER	0.21	8.4
M109	0.20	7.8
SW1271	0.0025	0.1

IN VITRO CELL PANEL SCREENING OF BU-SYR ANALOGS

BU-SYR RESISTANT CELL LINES	"ACTIVE" ANALOGS ^A No. ACTIVE/No. TESTED
B16-F10	2/9
M109	3/9
A549	2/9
MOSER	3/9

^A IC50 RATIO < 4 WHEN BU-SYR IS > 4

Characteristics of Mammalian Cell Lines for Antitumor Activity Prescreen

Response to known cytotoxic agents

- High sensitivity
- Resistance
- Combination of
sensitive and
resistance cell lines

Purpose

- Detection of materials
present in low amounts
in fermentation cultures
 - Dereplication of known
antitumor agents
 - Detection of new chemotypes
active on resistant tumor cells
 - Detection of materials
specifically active on
particular cell lines
-

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GROWTH REGULATION APPROACHES

1. DIFFERENT MODE OF ACTION FROM CLASSICAL CYTOTOXICS
2. NON-TOXIC TO NORMAL CELLS AND PERHAPS TO CANCER CELLS ALSO

APPROACHES FOR DISCOVERING POTENTIAL GROWTH REGULATORY PRODUCTS

1. PROPERTIES OF CELLS IN TISSUE CULTURE
 - A. GROWTH REQUIREMENTS
 - B. STATUS OF DIFFERENTIATION
 - C. MALIGNANCY SUPPRESSION IN HYBRIDS
2. PROPERTIES OF TISSUES
 - A. PROPERTIES RESPONSIBLE FOR SUPPRESSION OF CELL DIVISION (E.G. BRAIN)
 - B. FACTORS RENDERING SOME ORGANS GOOD OR BAD "SOIL" FOR THE GROWTH OF METASTASES RESULTING FROM SPECIFIC CELL TYPES
3. MECHANISM OF ACTION
 - A. MIMIC INHIBITORY FACTORS
 - B. INTERFERE WITH STIMULATORY FACTORS
 - C. MIMIC "DIFFERENTIATION AGENTS" (R.A., PLANAR POLAR)

HETEROGENEITY

- OBJECTIVE OF LARGE BANK IS TO OBTAIN
REPRESENTATION OF AS MANY SUB-TYPES OF COLON
CARCINOMA CELLS AS POSSIBLE

- HIGH SUCCESS RATE DESIRABLE
REFLECTIVE OF WIDER REPRESENTATION OF COLON
CARCINOMA CELL TYPES?
MORE EFFICIENT ESTABLISHMENT OF THE SAME
CELL LINES?

- MODELS FOR SPECIFIC TRAITS LIKE GROWTH
CONTROL OR DRUG RESISTANCE, NOT MODELS OF
TUMORS

HISTOPATHOLOGICAL SPECIFICITY

1. NORMAL COLON IS DIFFERENT FROM NORMAL LUNG

2. IF CANCERS ARE REFLECTIVE OF NORMAL TISSUE WOULD EXPECT THE
REGULATION OF PROLIFERATION AND DIFFERENTIATION IN COLON CANCER TO
BE DIFFERENT FROM THAT OF LUNG CANCER

3. COMPLEXITY OF CELL TYPES IN NORMAL COLON ALSO SUGGESTS A COMPLEXITY
OF GROWTH REGULATION IN THE NORMAL CELLS WHICH IS LIKELY TO BE
REFLECTED IN MALIGNANT CELL TYPES

ESTABLISHED BANK OF COLON CARCINOMAS USING FEEDER LAYERS OF EMBRYONIC
MESENCHYMAL CELLS

1. SUCCESS RATE: 75%

PREVIOUS RATES: 10-15%

2. WIDE RANGE OF INDIVIDUAL CELL TYPES

MORPHOLOGICAL

KARYOTYPE

XENOGRAFT GROWTH AND DIFFERENTIATION

CLONING EFFICIENCY

BIOCHEMICAL AND IMMUNOCHEMICAL MARKERS

EXOGENOUS GROWTH FACTOR REQUIREMENTS

AUTOCRINE GROWTH FACTOR PRODUCTION

3. DIVIDED ARBITRARILY INTO 3 BROAD CATEGORIES BASED ON

BIOLOGICAL PROPERTIES

CELL LINE BANK

GROUP I: UNDIFFERENTIATED, RAPID PROLIFERATION IN VITRO
AND AS XENOGRAFTS (20%)

GROUP III: WELL DIFFERENTIATED, SLOW PROLIFERATION IN
VITRO AND AS XENOGRAFTS (40%)

GROWTH PROPERTIES: GROUP I

UNDIFFERENTIATED XENOGRAFTS (100%)

LITTLE CELL CONCENTRATION DEPENDENCE OF GROWTH

ANCHORAGE DEPENDENT (10^3 - 10^5 CELLS)

ANCHORAGE INDEPENDENT (10^2 - 10^3 CELLS)

LITTLE DEPENDENCE ON FBS

PLATING EFFICIENCY 2% - 10%

NO RESPONSE TO EXOGENOUS FACTORS IN FBS

GROWTH PROPERTIES: GROUP III

WELL DIFFERENTIATED XENOGRAPHS (50%)

CELL CONCENTRATION DEPENDENCE OF GROWTH

ANCHORAGE DEPENDENT ($10^3 \angle 10^4$ CELLS)

ANCHORAGE INDEPENDENT ($10^4 - 5 \times 10^4$ CELLS)

DEPENDENCE OF FBS

PLATING EFFICIENCY AT 2% = 0

RESPONSE TO EXOGENOUS FACTORS IN FBS

PROPERTIES OF ADENOMAS

1. VILLOUS

A. HIGH PRE-MALIGNANT RISK

B. COMPLETELY INDEPENDENT OF EGF

2. TUBULAR

A. LOW RISK

B. EGF RESPONSIVE

ARE THE ADENOMAS REFLECTIVE OF GROWTH REGULATION STYLES IN COLON
CARCINOMA?

NON-MALIGNANT CELL LINES

1. CULTURED LINES OF NORMAL COLON NOT OBTAINED
2. PERMANENT LINES DEVELOPED FROM VILLOUS ADENOMAS AND FROM TUBULAR POLYPS.
 - A. DO NOT GROW WITH ANCHORAGE INDEPENDENCE.
 - B. DO NOT GROW IN ATHYMIC NUDE MICE
 - C. NO HISTOLOGICAL EVIDENCE OF CARCINOMA
 - D. APPEAR TO BE IMMORTAL, BUT OTHERWISE NON-MALIGNANT BY ANY IN VITRO CRITERION AVAILABLE

PROBLEMS FACING DETERMINATION OF GROWTH REQUIREMENTS FOR COLON
CARCINOMA

1. IN VITRO GROWTH CONTROLS REFLECT IN VIVO GROWTH CONTROLS
2. INTRA AND INTER-TUMORAL HETEROGENEITY OF MALIGNANT CELL TYPES MAKE THE POSSIBILITY OF MODELING HUMAN TUMORS IN VITRO VERY REMOTE

PROBLEMS IN DETERMINING GROWTH REQUIREMENTS CON'T

1. NEW TECHNOLOGIES FOR ASSESSING GROWTH RELATED MOLECULES IN WHOLE TISSUES AND TISSUE SECTIONS - ALSO APPLICABLE TO PRIMARY TISSUE CULTURES
2. DEVELOPMENT OF A BANK OF CELL LINES TO MODEL FOR INDIVIDUAL TRAITS OF COLON CARCINOMA RATHER THAN WHOLE TUMORS
3. DESIRABLE PROPERTIES
 - A. HIGH RATE OF SUCCESSFUL CELL LINE DEVELOPMENT FROM SPECIMENS ATTEMPTED
 - B. WIDE VARIETY OF CELL TYPES
 - C. CAPABLE OF GROWTH AS XENOGRAFTS
 - D. CAPABLE OF GROWTH IN CHEMICALLY DEFINED MEDIUM

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EGF RESPONSE IN GROUP III HUMAN COLON CARCINOMA CELLS

<u>CELL LINE</u>	<u>MEDIUM SUPPLEMENTATION</u>	<u>CELL NUMBER($\times 10^6$)</u>
GEO	TRANS, IN, EGF	4.8
	TRANS, IN	0.5
	TRANS, EGF	2.8
	IN, EGF	0.1
FET	TRANS, IN, EGF	1.8
	TRANS, IN	1.4
	TRANS, EGF	0.1
	IN, EGF	1.1
CBS	TRANS, IN, EGF	5.3
	TRANS, IN	3.1
	TRANS, EGF	0.8
	IN, EGF	0.3

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EGF BINDING TO HUMAN COLON CARCINOMA CELL LINES

GROUP	I			III		
CELL LINE	RKO	HCT 116	C	GEO	PET	CBS
RECEPTORS (PMOLES/MG DNA)	0.05+0.01	0.41+ 0.01	0.38+.03	1.03+.01	1.70+0.30	2.80+0.17
APPARENT K_D (NM)	0.24+0.10	0.15+0.02	0.17+0.01	0.17+0.01	0.28+0.05	0.30+0.01
NUMBER OF EXPTS.	3	3	1	2	3	3

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EGF ACTIVITY IN CONDITIONED MEDIA FROM A VARIETY OF CELL LINES (SERUM)

<u>CELL LINE</u>	<u>NG COMPETING ACTIVITY</u> MG PROTEIN
FET	9.5
CBS	11.1
MOSER	16.7
HCT 116	28.5

DIRECTIONS

1. WHAT IS THE BASIS FOR EGF INDEPENDENCE IN GROUP I CELLS?
2. IS THE GROUP I GROWTH REGULATORY STYLE REFLECTIVE OF A SUB GROUP OF COLON CARCINOMA CELL TYPES IN TUMORS?
3. DOES THIS GROWTH REGULATORY STYLE OFFER AN AVENUE OF THERAPEUTIC INTERVENTION?

MECHANISM OF ACTION OF GROWTH INHIBITORY MOLECULES

1. RETINOIDS
2. N,N-DIMETHYLFORMAMIDE (DMF)
3. TGF β

SIMILARITY OF EFFECTS

1. MORPHOLOGICAL - SATURATION DENSITY
2. INDUCTION OF EXTRACELLULAR MATRIX COMPONENTS
3. INHIBITION OF MITOGENIC SIGNALS
4. RAPID REPRESSION OF ONCOGENE EXPRESSION

ARE THERE MECHANISTIC SIMILARITIES WHICH WOULD ALLOW FOR THE IDENTIFICATION OF KEY MOLECULAR EVENTS?

CAN WE DEVELOP SYNTHETIC CHEMICALS OR IDENTIFY NATURAL AGENTS TO MIMIC THESE EVENTS W/O TOXICITY AND WITH HIGH POTENCY?

CHALLENGES

1. BIOLOGICAL AGENTS ARE RELATIVELY EXPENSIVE IN TERMS OF PRE-CLINICAL DEVELOPMENT - E.G. PROTEIN FACTORS WILL REQUIRE LARGE INVESTMENT TO OBTAIN ADEQUATE SUPPLIES FOR TESTING (CLINICAL SUPPLIES ALSO DIFFICULT)
2. THE BASIS FOR PRE-CLINICAL EVALUATION IS UNKNOWN
WHAT TESTING WILL JUSTIFY COMMITMENT TO THE INVESTMENT?

ALTERNATIVE STRATEGIES FOR THE DISCOVERY OF ANTINEOPLASTIC AGENTS

- COMPOUND-DRIVEN STRATEGY



- MECHANISM-BASED STRATEGY



- DISEASE-SPECIFIC STRATEGY

SCREENING OF TRANSITION-METAL COMPLEXES FOR ANTITUMOR ACTIVITY

- BASIS FOR SELECTION OF TRANSITION-METALS
 - ANTITUMOR ACTIVITY OF CISPLATIN AND RELATED PLATINUM COMPLEXES
 - PHARMACOLOGIC ACTIVITY REPORTED FOR GOLD COMPLEXES
 - EXPERIENCE IN GOLD CHEMISTRY AND ACCESS TO A SPECTRUM OF GOLD COMPLEXES
 - STRUCTURAL SIMILARITY OF GOLD(III) AND PLATINUM(II) COMPLEXES

SCREENING OF TRANSITION-METAL COMPLEXES FOR ANTITUMOR ACTIVITY

- COMPOUNDS SELECTED FOR SCREENING
 - PLATINUM COMPLEXES WERE NOT SCREENED
 - UNIQUE TRANSITION METALS WHICH HAVE NOT BEEN WIDELY TESTED I.E.

OSMIUM (II,III,IV), IRIDIUM (I,III,IV),
RHODIUM (I,II), RUTHENIUM (II,III,IV),
PALLADIUM (II), COBALT (III),
CHROMIUM (III), COPPER (I), NICKEL (II),
SILVER (I)

- PHOSPHINOGOLD (I) AND GOLD (III)
COMPLEXES BASED ON PRELIMINARY
OBSERVATIONS OF CYTOTOXIC PROPERTIES

SCREENING OF TRANSITION-METAL COMPLEXES FOR ANTITUMOR ACTIVITY

- BIOLOGICAL ASSAYS SELECTED FOR SCREENING
METAL COMPLEXES
 - CYTOTOXICITY PRESCREEN
 - CHEMOSENSITIVE MURINE TUMOR MODEL FOR
PRIMARY SCREENING

IP P388 LEUKEMIA

SCREENING OF TRANSITION-METAL COMPLEXES FOR ANTITUMOR ACTIVITY

- DETAILED EVALUATION OF LEADS IDENTIFIED IN PRIMARY SCREEN
 - A SPECTRUM OF TRANSPLANTABLE MURINE TUMOR MODELS
 - HUMAN TUMOR CLONOGENIC ASSAY
 - MECHANISM-OF-ACTION STUDIES
 - RESISTANT SUBLINES
 - CELL CYCLE PROGRESSION
 - MACROMOLECULAR SYNTHESIS
 - ASSAYS FOR DNA DAMAGE
 - INTERACTION WITH DNA IN VITRO
 - MITOCHONDRIAL EFFECTS

SCREENING OF TRANSITION-METAL COMPLEXES FOR ANTITUMOR ACTIVITY

- CRITERIA FOR IDENTIFYING TRANSITION-METAL COMPLEXES FOR DEVELOPMENT TO CLINICAL TRIAL
 - NOVEL STRUCTURE
 - SIGNIFICANT ACTIVITY IN AT LEAST THREE ANIMAL TUMOR MODELS
 - ACTIVITY IN A TUMOR MODEL DISTANT FROM SITE OF DRUG ADMINISTRATION
 - NOVEL MECHANISM OF ACTION

DISCOVERY OF DIPHOS GOLD COMPLEXES AS LEADS

- OVER 300 GOLD (I) AND GOLD (III) COMPLEXES WERE SYNTHESIZED AND SCREENED FOR ACTIVITY IN IP P388 LEUKEMIA
 - A LARGE PROPORTION OF GOLD COMPLEXES, PARTICULARLY PHOSPHINOGOLD COMPLEXES, HAD REPRODUCIBLE BUT MODEST ACTIVITY (30-50% ILS) IN P388 LEUKEMIA

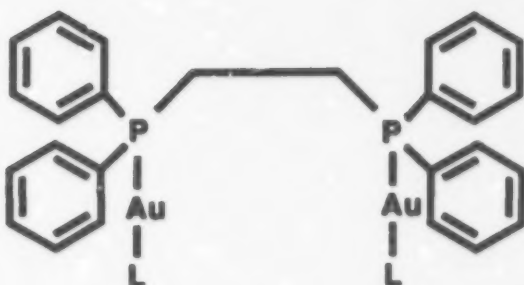
DISCOVERY OF DIPHOS GOLD COMPLEXES AS LEADS

- COMPOUNDS RELATED TO AURANOFIN, I.E. $R_3P-Au-L$, HAD GOOD ACTIVITY IN IP P388 (70-90% ILS) BUT HAD NO ACTIVITY IN OTHER TUMOR MODELS
- ONE CLASS OF COMPLEXES BEARING DIPHOS AND CLOSELY RELATED BISPHOSPHINES HAD GOOD ACTIVITY IN IP P388 (90-120% ILS) AND ACTIVITY IN OTHER TUMOR MODELS AS WELL

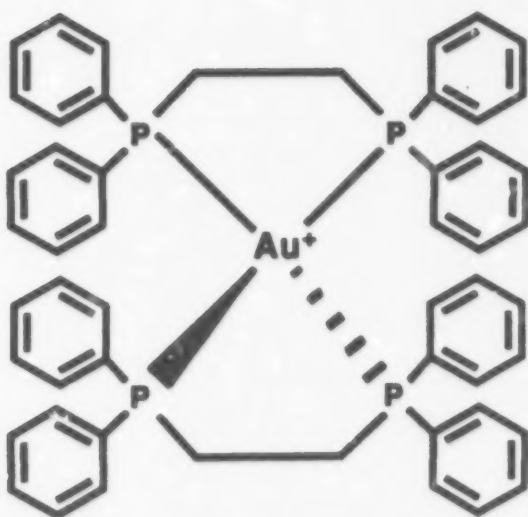
DIPHOS GOLD COMPLEXES WITH ANTITUMOR ACTIVITY



DIPHOS



**LINEAR
DIPHOS
BIS[GOLD(I)]
COMPLEX**



**TETRAHEDRAL
BIS(DIPHOS)
GOLD(I)
CATION**

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Table 2
Spectrum of Activity of $[\text{Au}(\text{dppe})_2]\text{Cl}$ in
Transplantable Murine Tumors

Tumor Model	Response of Tumor to $[\text{Au}(\text{dppe})_2]\text{Cl}$ ^a
<u>ip Tumors</u>	
P388 leukemia	++ ^b
M5076 reticulum cell sarcoma	++
B16 melanoma	+
L1210 leukemia	+
Colon carcinoma 26	-
B16 melanoma (F10 subline)	-
<u>iv Tumors</u>	
P388 leukemia	-
<u>sc Tumors</u>	
Mammary adenocarcinoma 16/c	+
Mammary adenocarcinoma 13/c	-
ADJ-PC6 plasmacytoma	*
M5076 reticulum cell sarcoma	*
Colon carcinoma 26	-
Colon carcinoma 07/A	-

^a ip administration

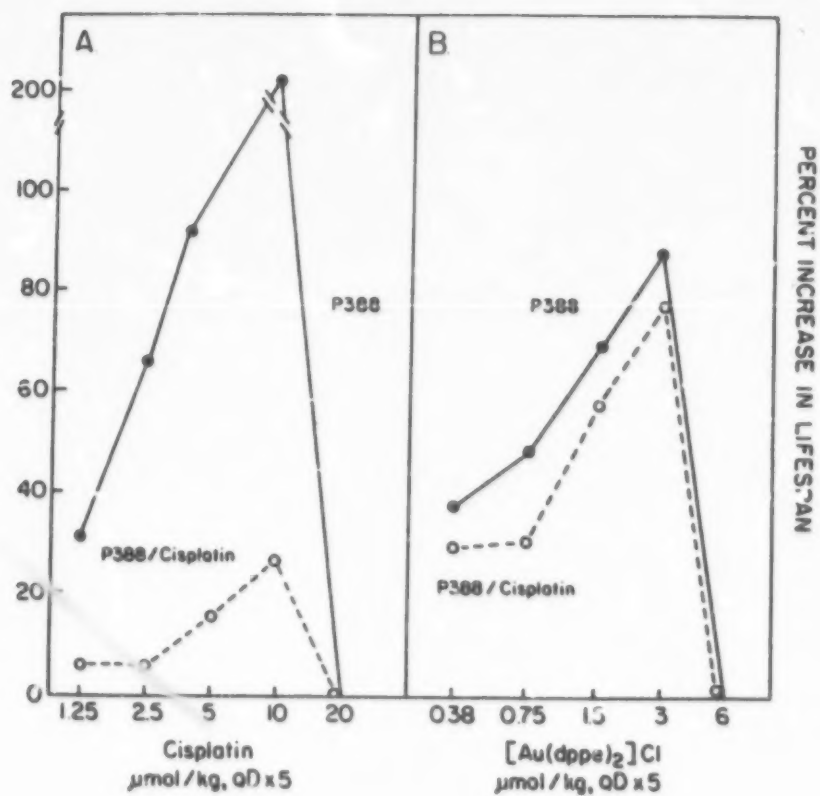
^b ++ indicates $\geq 50\%$ ILS in ip tumors and $\geq 90\%$ tumor growth inhibition in sc tumor models

+ indicates $\geq 30\%$ ILS in ip tumors and $\geq 75\%$ tumor growth inhibition in sc tumor models

* indicates unreproducible activity

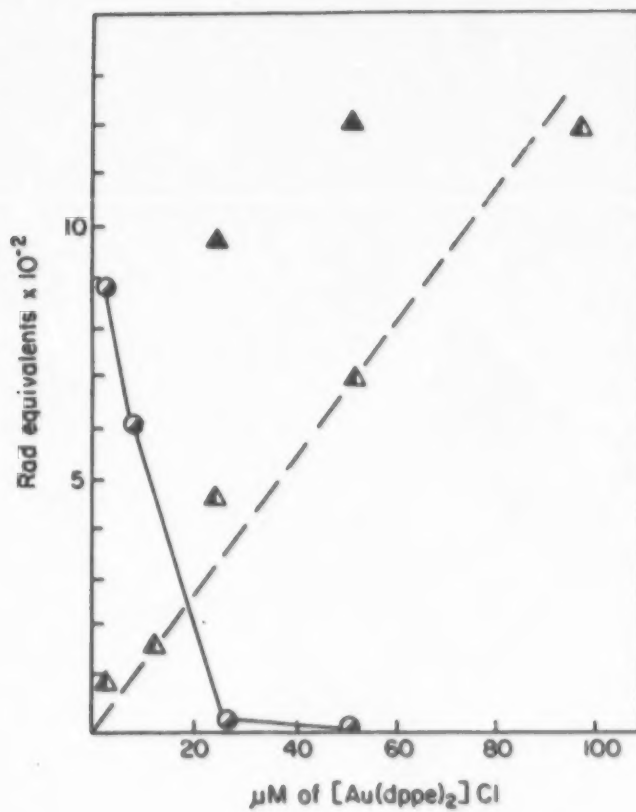
- indicates $< 30\%$ ILS in ip tumors and $< 75\%$ tumor growth inhibition in sc tumor models

Figure 4



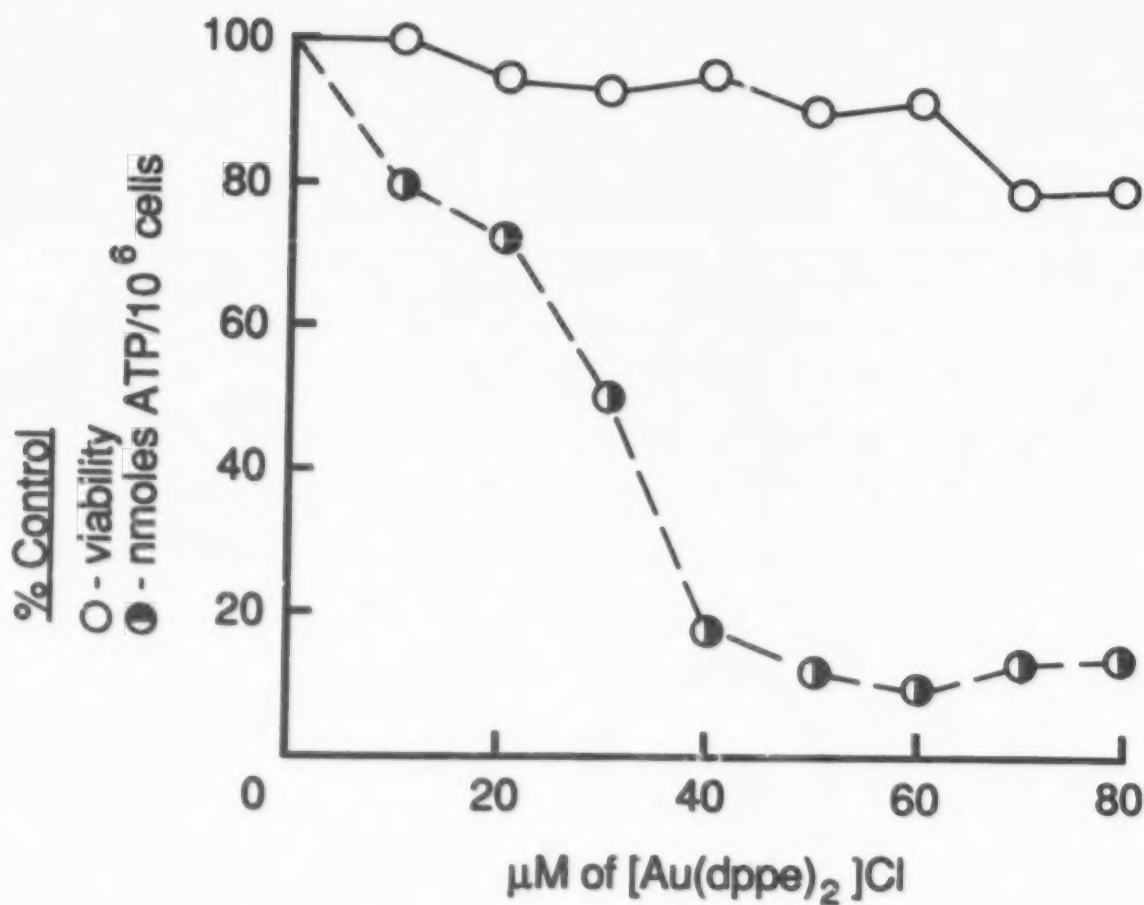
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Figure 6



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DEPLETION OF ATP IN P388 LEUKEMIA CELLS BY TETRAHEDRAL BIS(DIPHOS) GOLD(I)



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COMPOUND-DRIVEN STRATEGY

- SUMMARY

- IN VIVO SCREEN → TUMOR CELL SELECTIVITY
- NOVEL STRUCTURES → NOVEL MECHANISMS
- CELLULAR/MOLECULAR PHARMACOLOGY →
2ND GENERATION COMPOUNDS

MECHANISM-BASED ANTINEOPLASTIC DRUG DISCOVERY

- IDENTIFY BIOCHEMICAL TARGETS THAT ARE
RELEVANT AND/OR RATIONAL, I.E.
 - BINDING TO DNA AND/OR PRODUCTION OF DNA
DAMAGE
 - INHIBITION OF DNA DAMAGE REPAIR
 - INTERFERENCE WITH DNA TOPOISOMERASES
 - SPECIFIC ENZYMES INVOLVED IN NUCLEOTIDE
BIOSYNTHESIS
 - ONCOGENE PRODUCTS
 - STEPS INVOLVED IN ANGIOGENESIS, INVASION,
ETC.

**MECHANISM-BASED ANTINEOPLASTIC DRUG
DISCOVERY**

- DEVISE SELECTIVE SCREENS FOR IDENTIFICATION OF LEADS
 - SUBCELLULAR ASSAYS INVOLVING BINDING, ANTAGONISM, ENZYME INHIBITION, DAMAGE TO MACROMOLECULES, ETC.
 - CELLULAR ASSAYS SPECIFIC FOR A CHOSEN BIOCHEMICAL TARGET
 - MICROBIAL ASSAYS USING MUTANTS OR rDNA TECHNIQUES
 - IN VIVO ASSAYS SPECIFIC FOR THE DESIRED PATHWAY

MECHANISM-BASED ANTINEOPLASTIC DRUG DISCOVERY

- IDENTIFY COMPOUNDS WHICH INTERFERE WITH TARGET
 - SCREENING WITH SPECIFIC ASSAYS
 - DRUG DESIGN BASED ON ENZYME MECHANISM, AGONIST STRUCTURE OR SAR OF KNOWN INHIBITORS
- DETERMINE WHETHER LEAD COMPOUNDS HAVE APPROPRIATE PHARMACOLOGIC PROPERTIES
 - CYTOTOXICITY ASSAYS
 - ANIMAL TUMOR MODELS
 - HUMAN TUMORS IN VITRO/IN VIVO
 - CLINICAL TRIALS

TOPOISOMERASES AS THE TARGET OF A MECHANISM-BASED APPROACH

- IDENTIFICATION OF TOPOISOMERASES AS A TARGET FOR ANTINEOPLASTIC DRUGS
 - INHIBITORS OF DNA GYRASE (PROKARYOTIC DNA TOPOISOMERASE II) ARE AMONG THE MOST EFFECTIVE ANTIMICROBIAL AGENTS
 - TOPOISOMERASE II HAS BEEN IDENTIFIED AS BEING INVOLVED IN THE ACTION OF DNA BINDING DRUGS WITH ANTINEOPLASTIC ACTIVITY (I.E. ANTHRACYCLINES, MITOXANTRONE, AMSACRINE, ELLIPTICINES, ETC.)
- TOPOISOMERASES AS TARGETS
 - EPIPODOPHYLLOTOXINS AND CERTAIN ANTHRACYCLINES PRODUCE SIMILAR EFFECTS ON CELLS AND SUBCELLULAR SYSTEMS WITH NO APPARENT INTERACTION WITH DNA
 - CAMPTOTHECIN WAS DISCOVERED TO SPECIFICALLY INTERACT WITH MAMMALIAN DNA TOPOISOMERASE I
 - TOPOISOMERASES HAVE BEEN IDENTIFIED AS KEY ENZYMES INVOLVED IN GENE REPLICATION, REGULATION AND (REPAIR?)

**TOPOISOMERASES AS THE TARGET OF A
MECHANISM-BASED APPROACH**

- **USE OF SPECIFIC ASSAYS TO DISCOVER NEW
TOPOISOMERASE INHIBITORS**
 - **SCREENING TO DISCOVER NEW STRUCTURAL
LEADS**
 - **NATURAL PRODUCT SOURCES (CELLULAR
ASSAYS)**

**MARINE EXTRACTS, PLANT PRODUCTS,
MICROBIAL PRODUCTS**
 - **SYNTHETIC AGENTS/PURE NATURAL
PRODUCTS (CELLULAR AND SUBCELLULAR
ASSAYS)**

**NOVEL CHEMICAL STRUCTURES,
KNOWN CYTOTOXINS WITH UNKNOWN
MECHANISMS**

- SOURCES OF NOVEL TOPOISOMERASE INHIBITORS
 - EVALUATION OF ANALOGS OF KNOWN INHIBITORS
 - EPIPODOPHYLLOTOXINS, CAMPTOTHECIN ANALOGS, QUINOLONES, INTERCALATORS, NOVOBIOCIN ANALOGS
 - DEVELOPMENT OF SAR TO DEFINE SUBSTRUCTURES WHICH INTERACT WITH THE ENZYMES
 - DEFINITION OF ENZYME MECHANISM
 - DESIGN, SYNTHESIS AND EVALUATION OF ENZYME-MECHANISM BASED INHIBITORS

TOPOISOMERASES AS THE TARGET OF A
MECHANISM-BASED APPROACH

- DEVELOPMENT OF ASSAYS TO EVALUATE TOPOISOMERASES AND TO SELECT INHIBITORS
 - SUBCELLULAR ASSAYS FOR INHIBITORS OF ENZYME ACTIVITY AND FOR INDUCERS OF "CLEAVABLE COMPLEX"

- DEVELOPMENT OF TOPOISOMERASE ASSAYS
 - CELLULAR BIOCHEMICAL ASSAYS FOR COMPOUNDS WHICH PRODUCE PROTEIN-CONCEALED DNA BREAKS, PROTEIN-DNA CROSS-LINKS
 - CELLULAR BIOLOGICAL ASSAYS UTILIZING MAMMALIAN OR MICROBIAL MUTANT CELL LINES

TOPOISOMERASES AS THE TARGET OF A
MECHANISM-BASED APPROACH

- IDENTIFICATION OF NOVEL INHIBITORS AS CANDIDATES FOR BIOLOGICAL EVALUATION
 - GREATER POTENCY AT THE ENZYME LEVEL
 - GREATER SELECTIVITY, I.E. LACK OF DIRECT INTERACTION WITH DNA

- IDENTIFICATION OF NOVEL TOPOISOMERASE INHIBITORS
 - MECHANISTIC DIFFERENCES
 - LESS REVERSIBLE INHIBITION
 - ACTIVITY AGAINST MUTANT TOPOISOMERASES
 - DNA SEQUENCE-SPECIFIC TOPOISOMERASE INHIBITORS

TOPOISOMERASES AS THE TARGET OF A
MECHANISM-BASED APPROACH

- SELECTION OF NOVEL TOPOISOMERASE INHIBITORS FOR DEVELOPMENT TO CLINICAL TRIAL
 - GREATER SELECTIVITY FOR TUMOR CELLS, I.E. BROADER THERAPEUTIC INDEX AND/OR GREATER TUMOR CELL KILL AT A MAXIMALLY TOLERATED DOSE
 - BROADER SPECTRUM OF ACTIVITY IN ANIMAL TUMOR MODELS

- SELECTION OF NOVEL INHIBITORS FOR CLINICAL DEVELOPMENT
 - LACK OF CROSS-RESISTANCE WITH ESTABLISHED INHIBITORS OF TOPOISOMERASE II
 - IMPROVED SPECTRUM OF TOXICITY TO NORMAL TISSUES, I.E. NON-MYELOSUPPRESSIVE TOPOISOMERASE II INHIBITOR

COMPOUND-DRIVEN APPROACH



- GOLD-DIPHOS COMPLEXES

MECHANISM-BASED APPROACH

- DNA REACTIVE AGENTS
- TOPOISOMERASES
- UBIQUITINATION
- LIPID METABOLISM



- GROWTH FACTORS
- ONCOGENE PRODUCTS
- PROTEIN KINASES
- ?



DISEASE-SPECIFIC APPROACH

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DISCOVERY AND EVALUATION OF POTENTIAL ANTINEOPLASTIC AGENTS AT THE UPJOHN COMPANY

OUTLINE

- I. Screening for cytotoxic antitumor agents from natural and synthetic sources
 - A. Fermentation screening
 - B. Synthetic screening
- II. Screening for angiogenesis inhibitors
- III. Classification of leads
- IV. Factors in determining priority of leads

- I. **Screening for cytotoxics**

- A. **Fermentation screening**

- cellular vs. biochemical approach
- necessity for *in vitro* screening
 - low titres of active compounds
 - economics

- screens evaluated over the past 20 yr:
 - microbial models:
 - phage induction
 - *E. coli* DNA repair mutants
 - yeast with damaged cell membrane
 - hypoxic *E. coli*
 - mammalian models:
 - astrocytoma
 - human tumor cell line batteries
 - L1210 mouse leukemia-growth inhibition
 - dropped-poor production of novel compounds, lack of specificity

- current screens for antitumor antibiotics
- microbial antimetabolite assay - reversal of growth inhibition by metabolite - supplemented media

<u>culture #</u>	<u>nutrient agar</u>	<u>synthetic agar</u>	<u>synthetic agar +</u>	
			<u>amino acids</u>	<u>pur/pyr</u>
1	0	27	0	26
2	0	29	27	0

1 = acivicin
2 = 5-azacytidine

- T. Corbett's 2-tumor disc-diffusion assay
 - collaboration with Wayne State Univ. (Corbett, Valeriote, Baker)
 - designed to detect agents specifically toxic to chemotherapy-refractory solid tumor cells
 - discriminates between agents which kill rapidly proliferating cells and agents which, by whatever mechanism, kill slow-growing solid tumor cells

- utilizes cells from a solid tumor (colon 38, panc 03, mamm 16c, or human lung) and from a leukemia (murine, L1210 or P388)
- primary explants (preserves drug-resistance characteristics)
- *in vitro* soft-agar cloning assay (measures cell survival; economical)
- procedure
 - culture solid tumor cells and leukemia cells in same dish
 - fermentation beers or isolates, solutions of compounds are pipetted onto paper discs and applied to agar surface
 - 2-3 wk incubation
 - read zones of growth inhibition around the disc

● possible results:



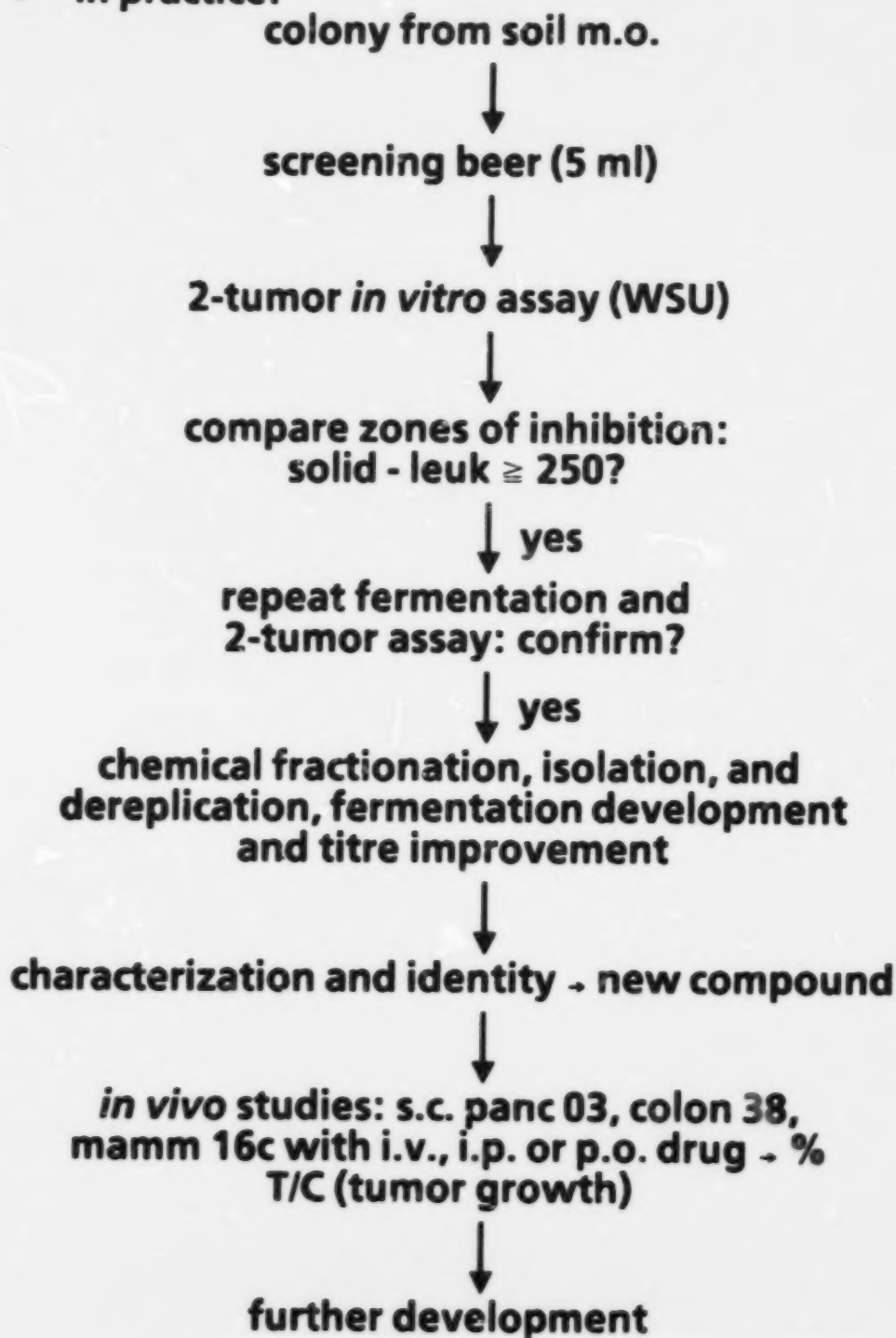
■ COLD-RECTAL TUMOR

△ LEUKEMIA

TWO TUMOR DISK ASSAY

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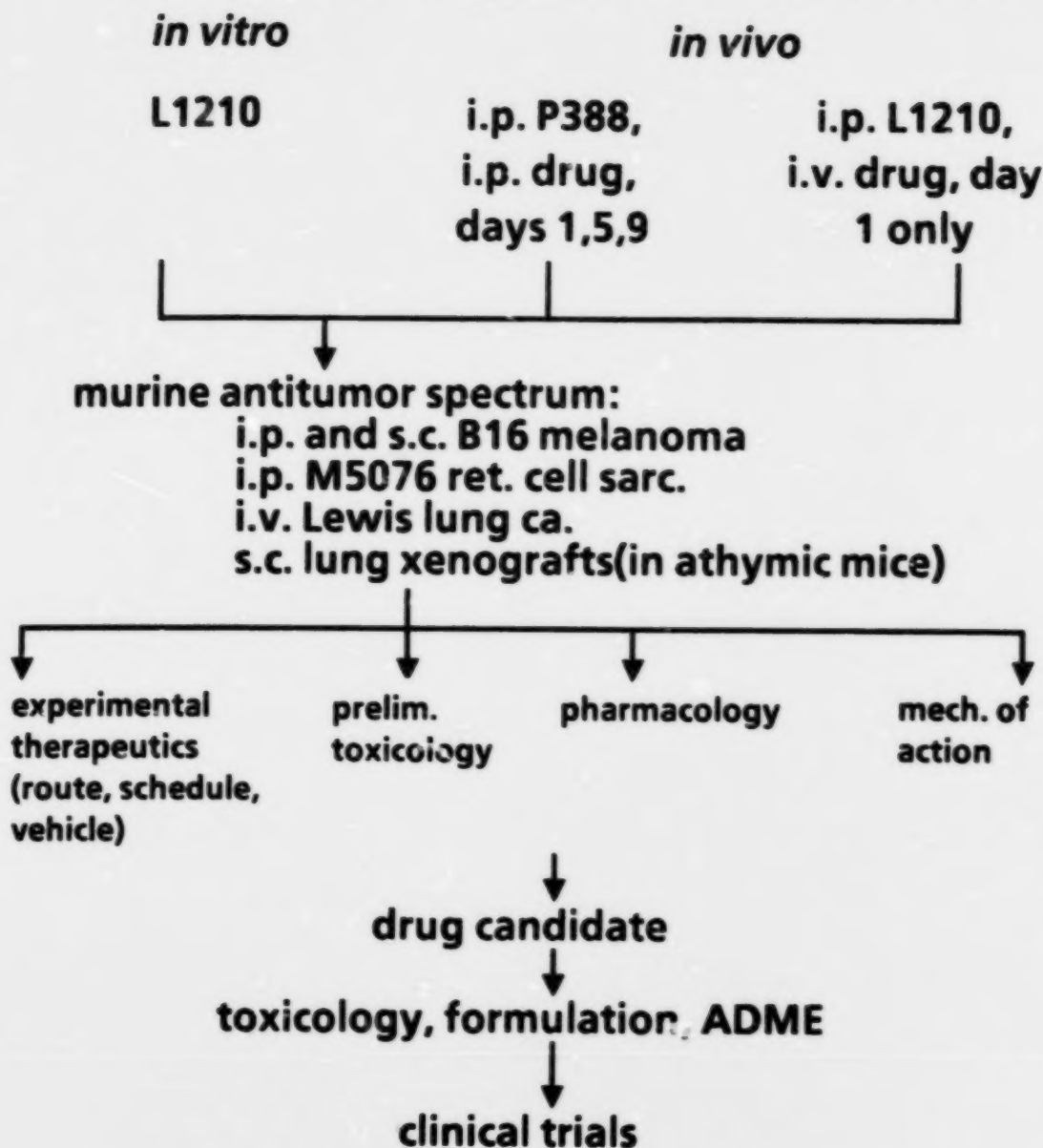
● in practice:



- **summary of 3 yr experience:**
 - **ca. 4500 beers screened**
 - **ca. 50 "confirmed active" beers with the desired solid tumor selectivity**
 - **several compounds isolated, most structures previously reported but the majority have never been recognized as antitumor agents**
 - **2-tumor assay is a vast improvement over L1210 leukemia as an isolation monitor**
 - **good correlation between *in vitro* and *in vivo* activity: intermediate fractionations do not have to be assayed *in vivo* → conservation of precious isolated material**
 - **assessment: fermentation screening using 2-tumor assay has good potential for discovery of novel structural/mechanistic classes**

B. Screening of synthetics

- **synthetic screening strategy depends on type of compound desired**
- **"random" screening-2-tumor assay**
- **synthetic analogs of the antibiotic, CC-1065:**

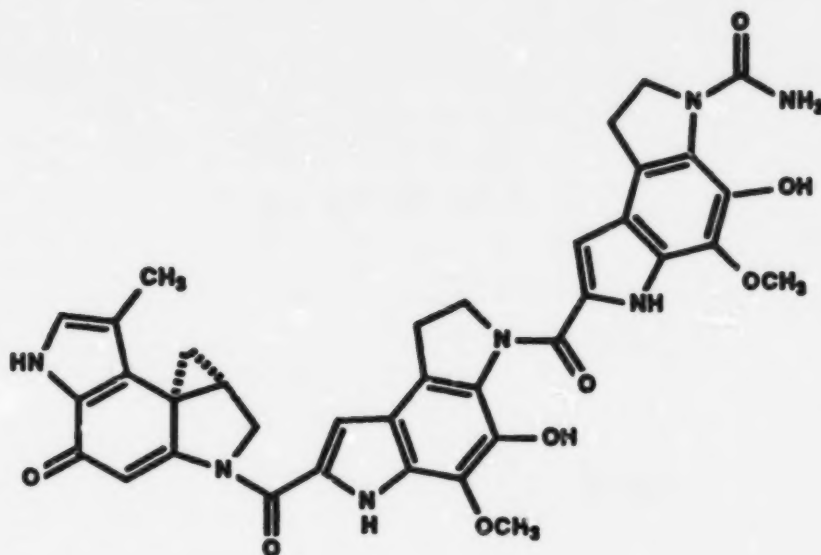


II. Screening for angiogenesis inhibitors

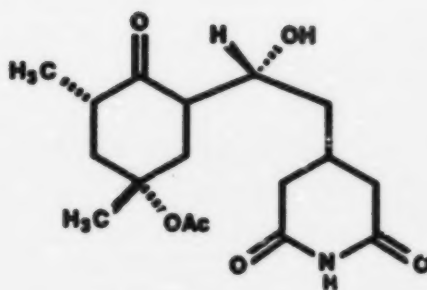
- collaboration with J. Folkman, Harvard
- cortisone or hydrocortisone + heparin or heparin fragment inhibits growth of new blood vessels in the chick embryo, rabbit cornea, and in experimental tumors in mice
- using steroid molecules provided by Upjohn, Folkman identified a new class of steroids lacking glucocorticoid or mineralocorticoid activity but possessing strong angiogenesis inhibiting activity = angiostatic steroids
- Folkman is screening steroid analogs prepared at Upjohn in the chick embryo chorioallantoic membrane (CAM) assay
- leads from the CAM assay are evaluated using mice bearing s.c.-M5076 sarcoma
- Harvard/Upjohn/NCI are currently collaborating on in-depth *in vivo* preclinical evaluation of a steroid analog prepared in our labs in combination with a hexasaccharide heparin fragment

SOURCES - NATURAL PRODUCTS

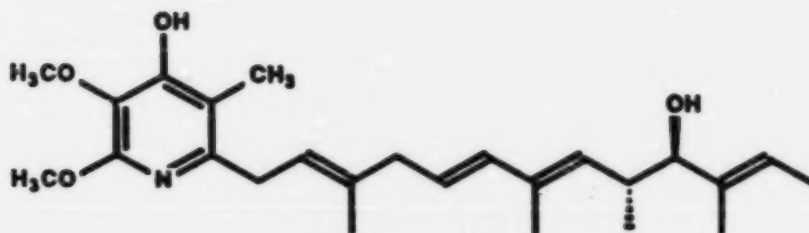
FERMENTATION DERIVED



CC-1065

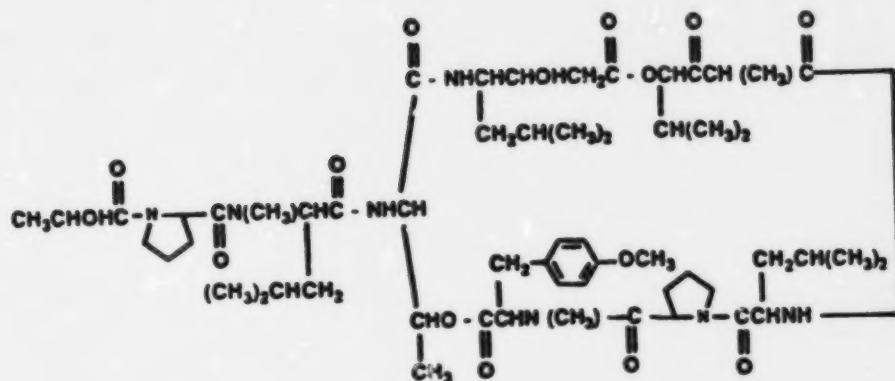


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PIERICIDIN A₁

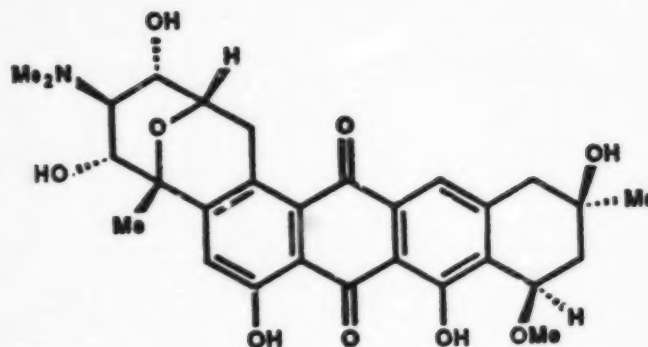
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Oc1ccc2c(c1)c3ccccc3[nH]2C4CCN(CS4)N[illegible]

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SOURCES - SYNTHETICS

SEMISYNTHETIC

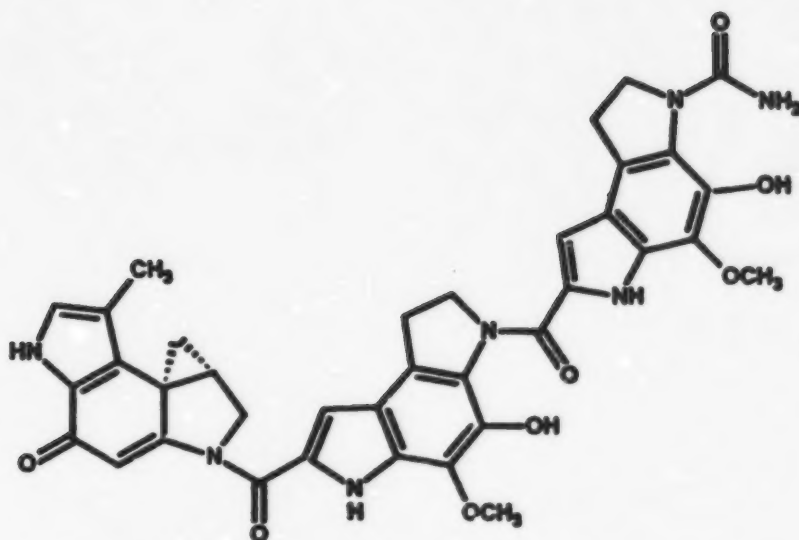


MENOGARIL

TOTALLY SYNTHETIC

- DE NOVO DESIGN
- ANALOGS OF NATURAL PRODUCTS
 - Cytotoxic CC-1065 Analogs
 - Angiostatic Steroids
- IMMUNOMODULATORS

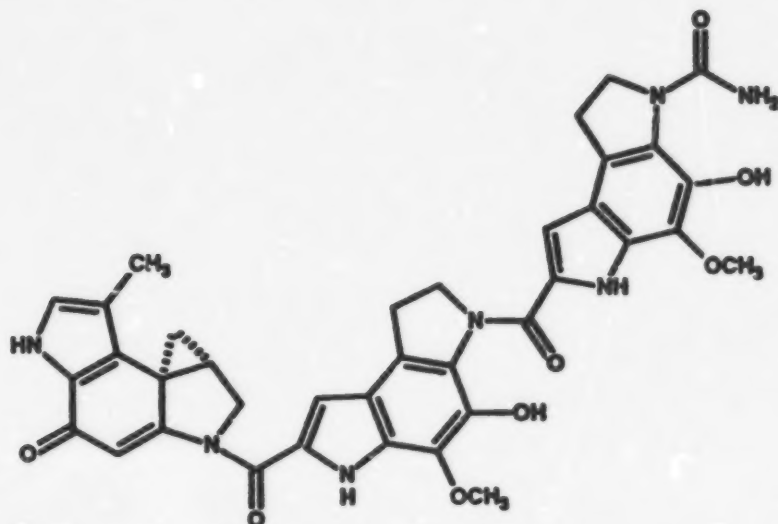
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CC-1065

- Isolated from Streptomyces zelensis
- Extremely potent in vitro cytotoxicity
- Broad spectrum antitumor activity
in mice at extremely low doses
- Unusual toxicity: delayed lethality in
mice at subtherapeutic doses
- Unique mechanism of action
 - Irreversible non-intercalative binding of
double-stranded B-DNA in the minor groove
 - Alkylation of the cyclopropyl ring at N-3 of
adenine
 - Selectivity for AT regions with two
consensus sequences

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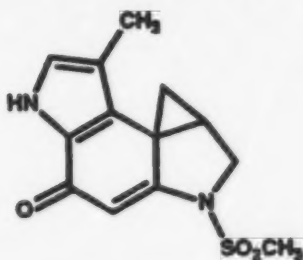


P388
(ip/ip)

T/C = 164
at 0.012 mg/kg

CC-1065 (U-56,314)

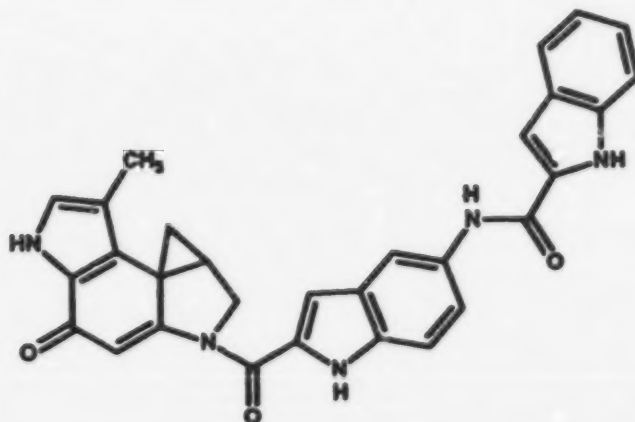
(L.J.Hanka, D.G.Martin, et al, J.Antibiot., 31,124(1978))



T/C = 160
at 25 mg/kg

U-62,736

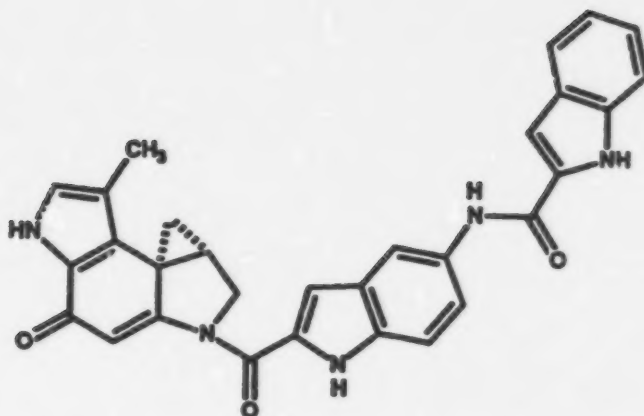
(W. Wierenga, JACS, 103, 5621(1981))



4/6 cures
at 0.050 mg/kg

U-68,415

(M.A.Warpehoski, et al, Proc. AACR, 26, 870(1985))

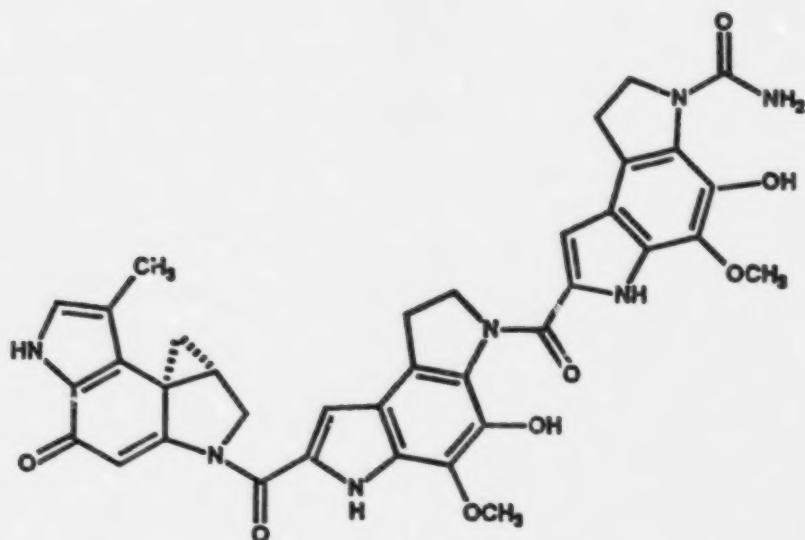


U-71,184

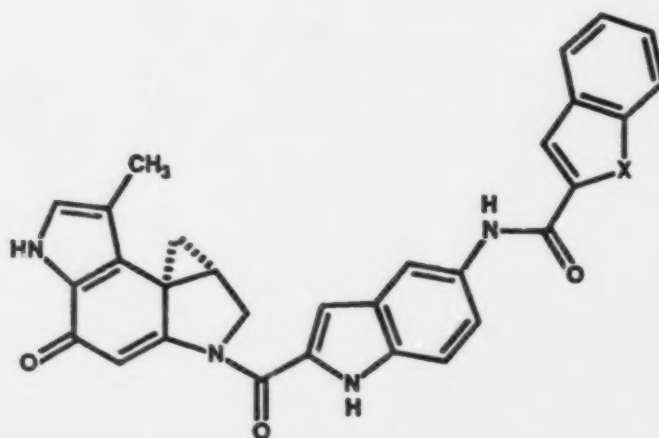
ADVANTAGES OF U-71,184

1. Novel and patentable antitumor agent
2. Effective against some transplanted human solid tumors
3. Curative in a number of tumor models
4. Extremely potent as well as single dose active
5. Active intravenously, the intended route of administration
6. Absence of delayed death phenomenon

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CC-1065 (U-56,314)



U-71,184 X = NH

U-73,975 X = O

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INCREASED LIFE-SPAN OF TUMOR-BEARING MICE

Tumor		U-73,975				CC-1065	
Site	System	%ILS (LTS) ^a	Dose ^b	Route	Schedule	%ILS	Dose ^b
i.p.	P388	(4/6-6/6)	25	i.p.	Days 1,5,9	70-111	30-100
i.p.	P388	130-135	50-100	i.v.	Day 1	20	200
i.v.	P388	(3/6-5/6)	100	i.v.	Day 1	62	100
i.p.	L1210	(3/6)	25	i.p.	Days 1,5,9	25-44	10-15
i.p.	L1210	75-100 (1/6)	100	i.v.	Day 1		
i.p.	L1210	156	50	i.v.	Days 1,8		
i.v.	L1210	143 (2/6)	50	i.v.	Day 1	46	100
s.c.	L1210	78	75	i.v.	Day 1		
i.p.	B16	194 (2/8)	10	i.p.	Days 1-9	59-81	10-100
i.p.	B16	68-93	100	i.v.	Day 1		
s.c.	B16	47	10	i.v.	Days 1-5	inactive	
i.p.	M5076	(7/8)	25	i.p.	Days 1,5,9		
i.p.	M5076	45	25	i.v.	Day 1		
i.v.	Lewis Lung	83 (2/8) - (5/8)	50	i.v.	Day 1	inactive	

a LTS = Long Term Survivors: 30 days for P388, L1210; 60 days for B-16, M5076, Lewis Lung

b In $\mu\text{g/kg/day}$; optimum dose

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Growth Inhibition of Advanced, Subcutaneously Implanted Tumors

Tumor System	U-73,975			
	% T/C ^a	Dose ^b	Route	Schedule
LX-1 Lung Carcinoma ^c	3***	100	i.v.	Day 1
Lewis Lung Carcinoma ^d	4***	75	i.v.	Day 1
B-16 Melanoma ^d	3***	70	i.v.	Day 1
M-5076 Sarcoma ^d	5-12***	80-100	i.v.	Day 1

a Mean tumor volume change of test group/vehicle controls x 100%, reporting optimum (lowest) T/C measured in a given experiment. *** p < 0.001, ** p < 0.01, * p < 0.05

b Optimum dose in µg/kg/day

c Human tumor xenograft in nude mice

d Murine tumor administered as a brei

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STUDIES LEADING TO NEW APPROACHES IN DRUG DISCOVERY/DEVELOPMENT

- **in transformation**
 - hormonal modulation by ras p21
- **in progression and metastases**
 - stabilization factors
- **in stasis or differentiation**
 - growth factors (TGF α antagonists)
 - angiogenesis (angiostatic steroids)
- **in immunology**
 - immunomodulators (bropirimine)

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THE ANTICANCER DRUG DISCOVERY PROGRAM
AT WARNER-LAMBERT/PARKE-DAVIS

1. INTRODUCTION: EXISTING SCREENING APPROACHES AND THEIR LIMITATIONS (R.C. JACKSON)
2. THE USE OF IN VIVO MURINE TUMOR SYSTEMS FOR SELECTION OF LEAD COMPOUNDS (W. R. LEOPOLD)
3. THE USE OF CELL BIOLOGY AND BIOCHEMICAL PHARMACOLOGY APPROACHES TO IDENTIFY NOVEL INTERCALATING AGENTS (J. S. SEBOLT)
4. OUTSTANDING QUESTIONS CONCERNING EXISTING SCREENING APPROACHES (R. C. JACKSON)
5. NEW APPROACHES TO DEVELOPMENT OF AGENTS WITH ACTIVITY AGAINST COLON CARCINOMA AND AGAINST PLEIOTROPICALLY RESISTANT TUMORS (W. D. KLOHS)

INTRODUCTION

EXISTING SCREENING APPROACHES IN THE WL/PD
ANTICANCER PROGRAM

1. IN VITRO PRESCREENING (L1210, HCT-8, OTHER)
2. IN VIVO PRESCREEN P388 (IN-HOUSE OR NCI)
3. SECONDARY IN VIVO SCREEN (MURINE SOLID TUMOR)
4. IN VITRO MURINE TUMOR PANEL
5. IN VIVO MURINE TUMOR PANEL
6. XENOGRAFT STUDIES
7. HUMAN TUMOR STEM CELL COLONY ASSAYS
8. BIOCHEMICAL SCREENS

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OUTSTANDING QUESTIONS CONCERNING THE EXISTING
SCREENING APPROACHES

1. CAN MOUSE TUMOR LINES IN VITRO PREDICT FOR RESPONSE
OF THE SAME MURINE TUMOR IN VIVO? CAN WE PREDICT:
EFFICACY?
POTENCY?
DRUG RESISTANCE?
2. IF THE ANSWER TO (1) IS "NO", CAN EX VIVO CLONOGENIC
ASSAY BE USED TO PREDICT RESPONSE?
3. DOES THE HUMAN TUMOR STEM CELL COLONY ASSAY PREDICT
DIFFERENTLY FROM THE USE OF ESTABLISHED HUMAN TUMOR
LINES?
4. DOES THE USE OF XENOGRAFTS TO IDENTIFY THE BEST COMPOUND
IN A SERIES PREDICT DIFFERENTLY FROM MURINE TUMORS?
5. CAN HUMAN TUMOR STEM CELL LINES IN VITRO PREDICT THE
SPECTRUM OF CLINICAL ACTIVITY?
E.G. SOLID TUMOR VS. ANTILEUKEMIC ACTIVITY?
BROAD SPECTRUM VS. NARROW SPECTRUM?
ACTIVITY AGAINST A PARTICULAR TUMOR CLASS?

OUTLINE FOR AN IN VITRO - IN VIVO TEST SYSTEM

1. Murine melanoma B16(B16) can be readily transferred from culture
to mice and vice versa
2. In vivo the tumor can be maintained ip or sc. Drug activity can
be monitored by lifespan assays or by growth delay, or ex vivo,
i.e. by in vivo treatment followed by excision of tumor, and
measurement of viable tumor cells by colony assays
3. In vitro the tumor cells can be maintained in monolayer and
colony assays can be performed in soft agar
4. An Adriamycin-resistant subline can also be grown in vitro
and in vivo

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MEASUREMENT OF MYELOSUPPRESSION BY CFU-C(GM) ASSAY

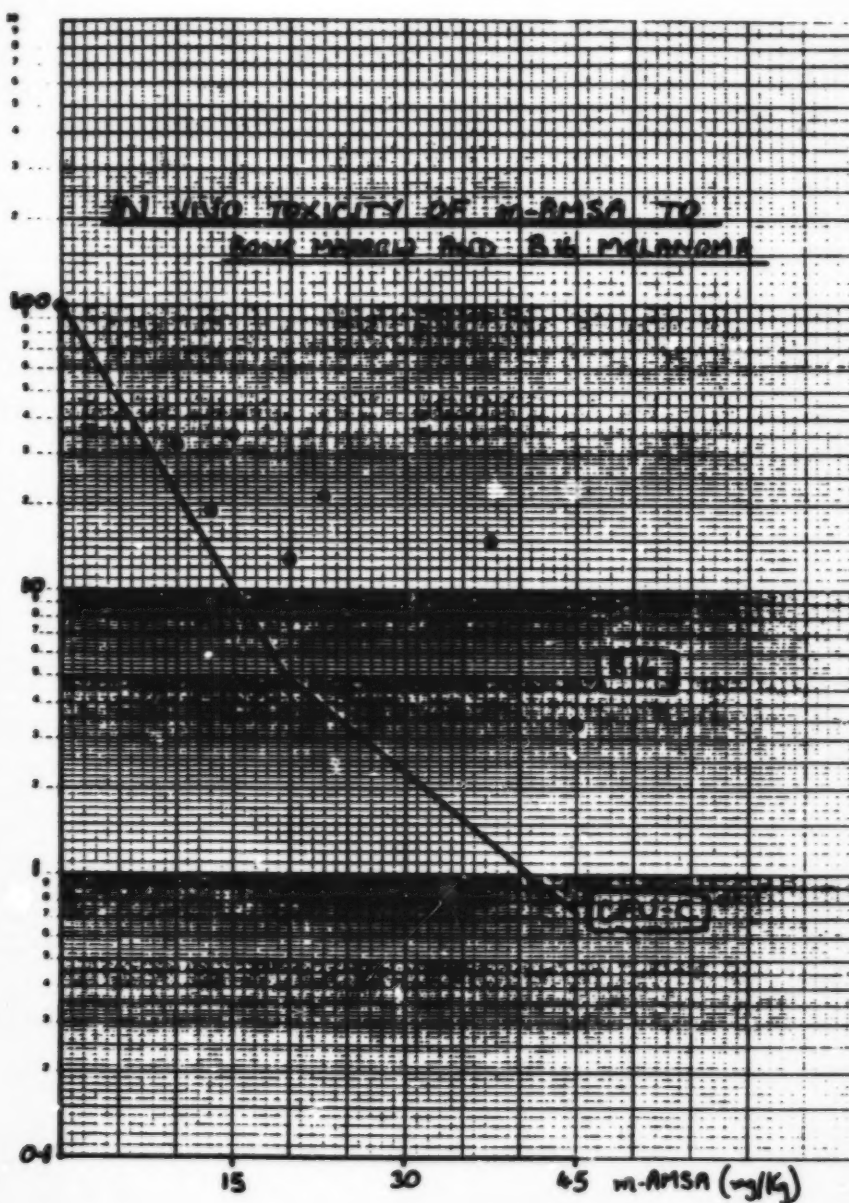
1. Bone marrow is removed from the femur of a B6C3F1 mouse by flushing with 0.7 ml of RPMI1640 medium, using a 20G x 1/2" needle. 5 million cells per femur are obtained.
2. 20,000 - 200,000 cells are plated into Fischer's medium containing 20% horse serum, 0.2% Dextrose agar (Difco), 10% L-829 cell conditioned medium, + penicillin/streptomycin (1 ml total volume).
3. Colonies are counted visually after 7 days at 37 degrees, 5% CO₂ in a humid incubator.

Control plating efficiency is c. 0.18%

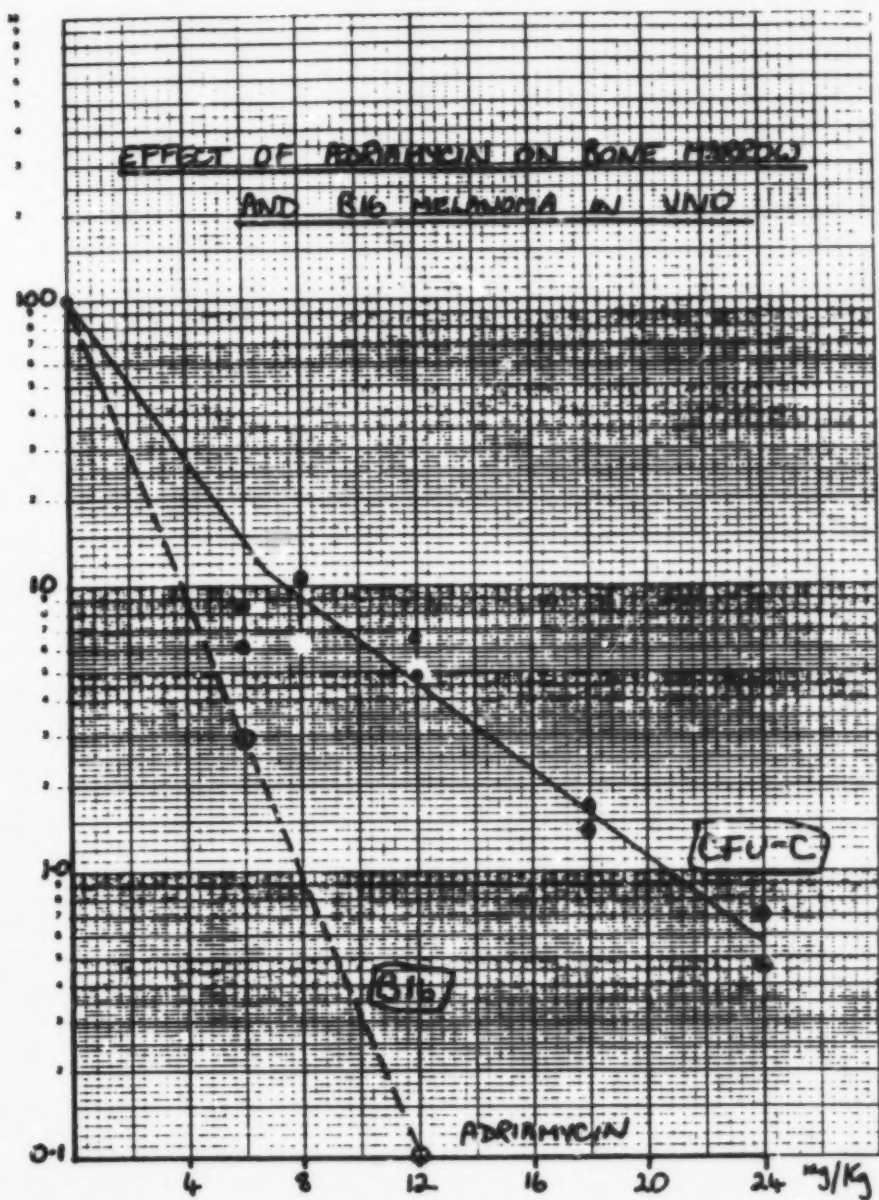
EXPT. RCJ 85/17

Treatment	Dose mg/kg	Cells/femur (% control)	P.E. (%)	CFU-C/ femur % control
Control		(100)	(100)	(100)
Adriamycin	8	70	14.5	10.2
"	8	70	14.4	10.1
"	12	67	10.1	6.8
"	12	21	24.2	5.1
Anthrone	20	45	13.0	5.8
"	20	58	15.4	8.9
"	30	42	6.3	2.6
"	30	25	9.2	2.3

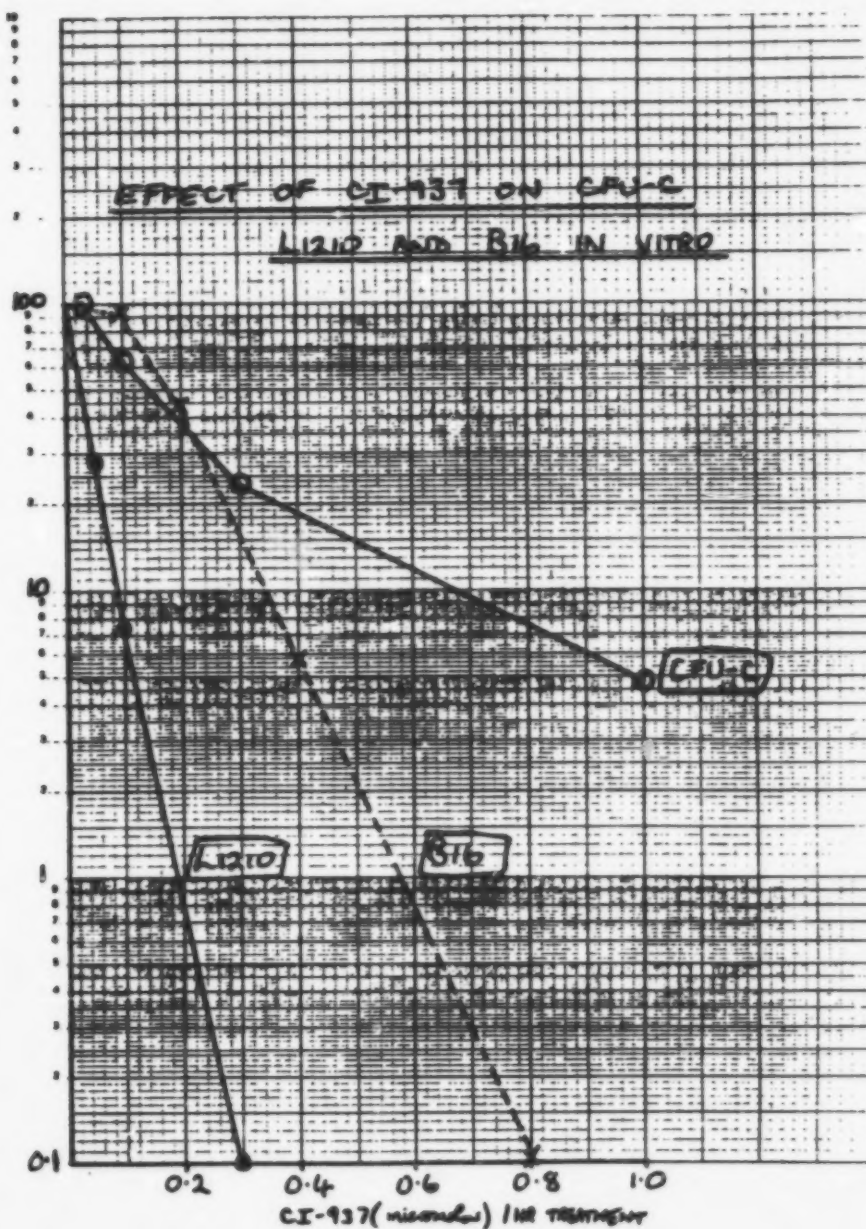
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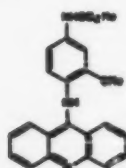
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BONE MARROW TOXICITY OF m-ANSA

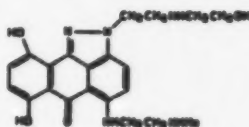
1. MTD for a single dose of m-ANSA is 30 mg/kg (ip).
 2. This gives 97.4% kill of CFU-G.
 3. Comparing with the in vitro data, this corresponds to treatment for 60 minutes at 1.04 micromolar.
 4. This value may be regarded as the in vitro MTD equivalent.
- What degree of cell kill does this concentration give for various tumour cell types?



(NCC 249902)

BONE MARROW TOXICITY OF CI-837

1. MTD for a single dose of CI-837 is 0.6 mg/kg.
2. This gives 78% kill of CFU-G.
3. Comparing with the in vitro data, this corresponds to treatment for 60 min at 0.33 micromolar.
4. This concentration of CI-837 gives > 99.9% kill of L1210 cells in vitro, and ~88% kill of B16 cells.



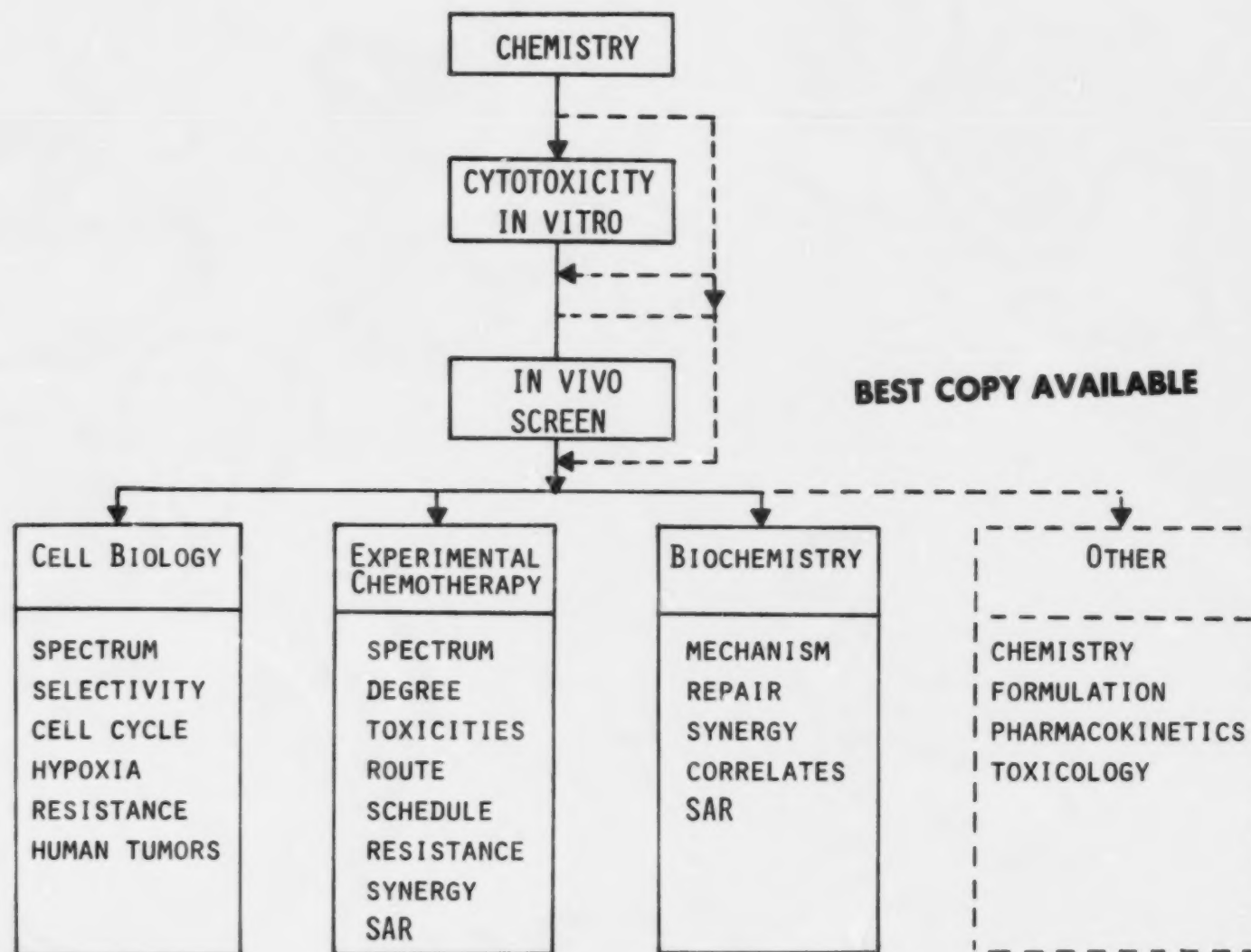
• DMC1

CONCLUSIONS

1. Based upon inhibition of CFU-G at the MTD, Adriamycin is more myelosuppressive than CI-837, and m-ANSA more than Adriamycin.
2. m-ANSA is less cytotoxic to B16 cells than to CFU-G.
3. For degree of cell kill > 1 log, Adriamycin is more cytotoxic to B16 cells than to CFU-G in vitro.
4. Adriamycin also gave a good therapeutic index against B16 in vivo.
5. CI-837 was more cytotoxic (in vitro and in vivo) to B16 than to CFU-G, and (in vitro) much more cytotoxic to L1210 cells than to CFU-G.

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ANTICANCER DRUG DISCOVERY AND DEVELOPMENT STRATEGY



IN VIVO TUMOR MODELS

SELECTION CRITERIA

1. DIVERSE BIOLOGY
2. LIMITED CHEMOSENSITIVITY
3. LOW IMMUNOGENICITY

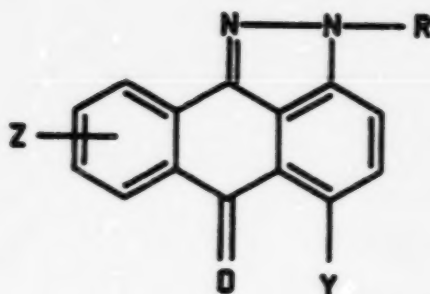
USE

1. IMPOSE PHYSIOLOGIC BARRIERS
2. DESIGN CURE OUT OF EXPERIMENT
3. DRUG SPECIFIC SCHEDULE
4. AT LEAST EIGHT MODELS

EVALUATION CRITERIA

1. FREQUENCY OF RESPONSE ($\geq 30\%$)
2. DEGREE OF RESPONSE
 - %ILS > 100 LIFESPAN
 - %T/C ≤ 10 GROWTH INHIBITION
 - T-C ≥ 10 GROWTH DELAY
(NET KILL > 1 LOG)

ACTIVITY OF SELECTED ANTHRAPHYRAZOLES
VS THE NCI TUMOR PANEL



AGENT	Z T/C				
	P388	L1210	B16	M5076	MX-1
109558	264	240 (1/6)	189	217	29
111257	242 (2/5)	188	183	209	5 (2/6)
113174	291 (6/6)	333 (3/6)	342 (6/10)	160	-98 (4/5)
111815	254 (4/5)	337 (4/5)	263 (4/10)	186	-100 (5/6)
113309	277 (6/6)	337 (3/6)	169	245 (6/10)	-78 (2/6)
113785	285 (5/6)	361 (4/6)	306 (9/10)	263 (5/10)	-100 (6/6)
ADR	211	147	238 (6/8)	216	-10
MITOX	312 (5/6)	361 (4/6)	265 (4/10)	260 (6/8)	6 (1/6)

ALL TESTS PER NCI STANDARD PROTOCOL.

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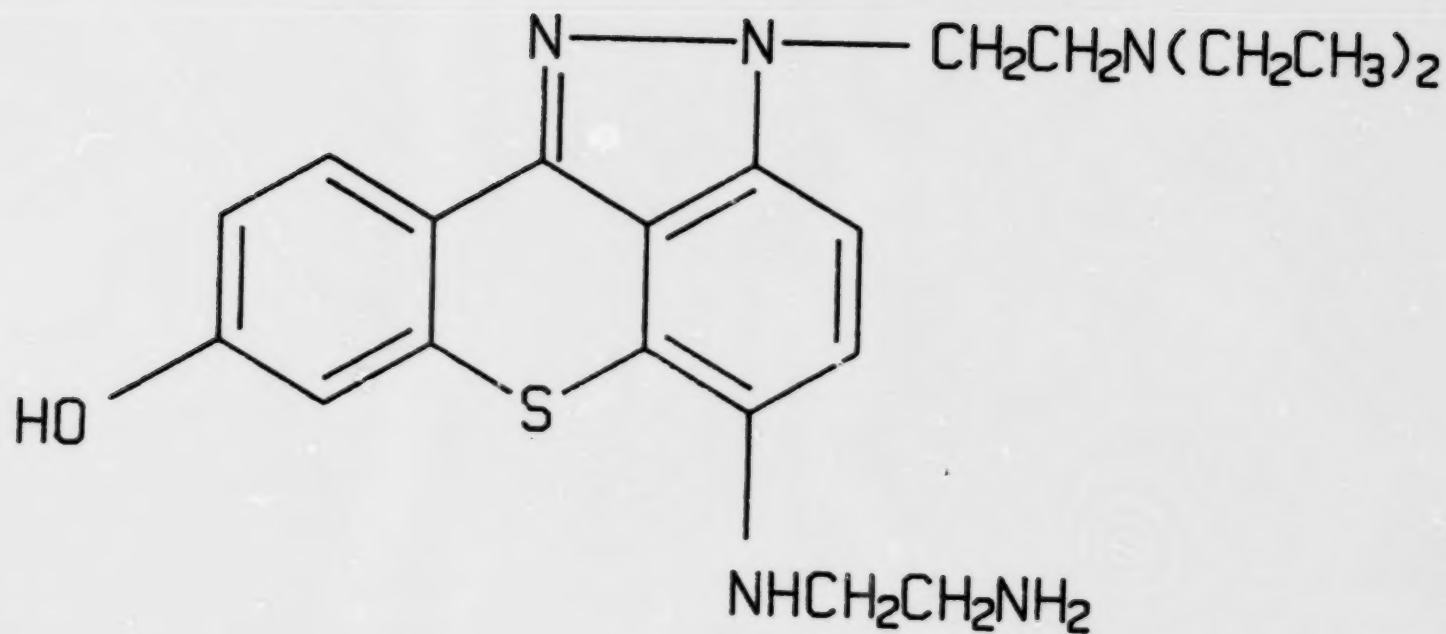
ACTIVITY OF SELECTED ANTHRAPHYRAZOLES
VS. SOLID TUMORS OF MICE

AGENT	GROWTH DELAY (RATING)		% T/C ROS
	MAMMARY 16c	COLON 11A	
109558	1.0 (-)	4.9 (-)	6 (AA)
111257	1.8 - 2.8 (-/+)	1.7 (-)	2 (AA)
113174	1.7 (-)		0 (AA)
111815	12.3 - 17.1 (++++)	8.9 (-)	7 (AA)
113309	7.8 - 12 (++/++++)	16.9 (++)	0 (AA)
113785	11.4 (++++)	9.2 (+)	0 (AA)
DOXORUBICIN	8 - 19 (++/++++)	19.8 (++)	0 (AA)
MITOXANTRONE	3.8 - 4.4 (++)	4.1 (-)	50 (-)

SC TUMORS

IV TREATMENT Q4DX3

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RANK ORDER OF ACTIVITY FOR SELECTED
BENZOTHIOPYRANOINDAZOLES IN
THE WL/PD TUMOR PANEL

<u>TUMOR</u>	<u>114214</u>	<u>114595</u>	<u>118484</u>	<u>120104</u>	<u>121373</u>
M16c	4	5	1	3	2
M13c	3	4	1	2	5
M17c	4	3	2	5	1
C11A	5	2	1	4	3
C36	3	2	1	5	4
—	—	—	—	—	—
MEAN	3.8	3.2	1.2	3.8	3.0

COMPARATIVE ACTIVITY OF PD 118,484 AND SELECTED
 REFERENCE COMPOUNDS AGAINST MOUSE SOLID TUMORS (SC)

<u>TUMOR SYSTEM</u>	<u>NET CHANGES IN TUMOR BURDEN (LOGS)</u>		
	<u>PD 118,484</u>	<u>ADRIAMYCIN</u>	<u>MITOXANTRONE</u>
MAMMARY 16C	- 2.5	- 2.5	+ 1.5
MAMMARY 17	- 5.8	ND	- 4.0
MAMMARY 13C	- 3.1	+ 0.6	- 1.1
COLON 36	+ 0.1	ND	+ 0.5
COLON 11A	- 2.7	- 0.8	+ 0.2
RIDGWAY OSTEOSARCOMA	8/10 CURES	9/10 CURES	+ 0.6
SCHEDULE	Q4HX3; Q4DX3 IV	Q4DX3 IV	Q4DX3 IV

BIOCHEMICAL CHARACTERISTICS OF PD 118484 COMPARED TO OTHER INTERCALATORS

<u>DRUG</u>	<u>DNA</u>	<u>RNA</u>	<u>OXYGEN CONSUMPTION</u>	<u>DNA BREAKS</u>	<u>DNA AFFINITY</u>
PD 118484	0.60	0.59	6.9	0.26	1.19
ADRIAMYCIN	2.05	2.10	144.1	0.14	3.87
MITOXANTRONE	0.50	0.60		0.84	
AMSACRINE	0.34	26.0		0.58	0.02

-
- INHIBITS BOTH DNA AND RNA SYNTHESIS WITH EQUAL POTENCY SIMILAR TO ADRIAMYCIN AND MITOXANTRONE BUT UNLIKE α -AMSA AND THE ANTHRAPHYRAZOLES.
 - HAS A LOW POTENTIAL TO FORM SUPEROXIDE RADICAL IN THE PRESENCE OF RAT LIVER MICROSOMES IN CONTRAST TO ADRIAMYCIN.
 - BINDS TIGHTLY TO DNA WITH AN ASSOCIATION CONSTANT SIMILAR TO OTHER TIGHT BINDING INTERCALATORS.
 - PRODUCES SINGLE AND DOUBLE STRAND DNA BREAKS WHICH ARE REPAIRED VERY SLOWLY SIMILAR TO ADRIAMYCIN BUT UNLIKE α -AMSA.

PREDICTION OF CLINICAL SOLID TUMOR ACTIVITY
FROM MURINE SYSTEMS

1. USE LEUKEMIAS ONLY AS PRE-SCREENS. EXCLUDE LEUKEMIA DATA FROM RESPONSE RATE ANALYSIS.
2. COUNT ONLY HIGHLY ACTIVE RESULTS.
 $ILS \geq 100\%$ IN LIFESPAN ASSAY.
 $T/S \geq 10\%$ IN GROWTH INHIBITION ASSAY.
 $T-C \geq 10$ DAYS IN GROWTH DELAY ASSAY.
3. TEST IN AT LEAST EIGHT SOLID TUMOR SYSTEMS.
4. LOOK FOR RESPONSE RATE $\geq 30\%$.

RESPONSE RATES OF MURINE SOLID TUMORS
TO THERAPEUTIC AGENTS

<u>DRUG</u>	<u>NO. OF SYSTEMS HIGHLY RESPONSIVE</u>	<u>RESPONSE RATE</u>
CYCLOPHOSPHAMIDE	7/11	64%
MELPHALAN	5/8	62%
ADRIAMYCIN	11/19	58%
CISPLATIN	8/16	50%
VINCRIStINE	5/12	42%
MITOXANTRONE	4/10	40%
ETOPOSIDE	2/5	40%
5-FLUOROURACIL	3/8	37%
BCNU	4/11	36%
BLEOMYCIN	3/9	33%
AMSACRINE	3/12	25%
MITOMYCIN-C	2/8	25%
6-MERCAPTOPURINE	2/8	25%
ACTINOMYCIN-D	2/9	22%
DAUNORUBICIN	1/5	20%
DIAZIQUNE	1/9	11%
CYTARABINE	0/8	0%

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RESPONSE RATES OF MURINE SOLID TUMORS TO THERAPEUTIC AGENTS

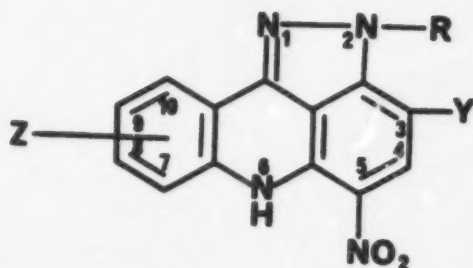
<u>DRUG</u>	<u>NO. OF SYSTEMS HIGHLY RESPONSIVE</u>	<u>RESPONSE RATE</u>
CI-958	8/11	73%
CYCLOPHOSPHAMIDE	7/11	64%
MELPHALAN	5/8	62%
ADRIAMYCIN	11/19	58%
CI-937	7/13	54%
CISPLATIN	8/16	50%
VINCRIStINE	5/12	42%
MITOXANTRONE	4/10	40%
ETOPOSIDE	2/5	40%
5-FLUOROURACIL	3/8	37%
BCNU	4/11	36%
BLEOMYCIN	3/9	33%
AMSACRINE	3/12	25%
MITOMYCIN-C	2/8	25%
6-MERCAPTOPURINE	2/8	25%
ACTINOMYCIN-D	2/9	22%
DAUNORUBICIN	1/5	20%
DIAZIQUONE	1/9	11%
CYTARABINE	0/8	0%

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CAN CELL BIOLOGY AND BIOCHEMICAL PHARMACOLOGY PLAY A PRIMARY ROLE IN ANTITUMOR DRUG DESIGN?

APPROACHES AVAILABLE:

- - - TESTING OF HUMAN SOLID TUMOR CELL LINES IN
TISSUE CULTURE.
- - - HUMAN PRIMARY TUMOR STEM CELL CLONOGENIC
ASSAYS.
- - - IN VITRO SCREENING AGAINST REFRACTORY TUMOR
CELL POPULATIONS:
 - A. HYPOXIC CELLS
 - B. NONCYCLING OR QUIESCENT CELLS
 - C. MULTIDRUG RESISTANT CELLS
- - - STANDARD BIOCHEMICAL ASSAYS TO MEASURE
INHIBITION OF MACROMOLECULAR SYNTHESIS.



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Growth inhibition of human solid tumor cell lines by the pyrazoloacridines

Compound	BREAST				COLON				LUNG	
	MCF-7		MB-231		HCT-8		WiDr		A549	
	IC ₅₀ ^a	Ratio ^b	IC ₅₀	Ratio	IC ₅₀	Ratio	IC ₅₀	Ratio	IC ₅₀	Ratio
PD 110,334	310	1.6	588	3.1	161	0.84	1528	8.0	579	3.0
PD 111,425	273	1.1	435	1.7	152	0.60	979	3.9	391	1.5
PD 113,377	139	0.30	267	0.57	60	0.13	1244	2.7	272	0.58
PD 114,245	118	0.30	206	0.52	67	0.17	607	1.5	255	0.64
PD 114,541	42	21	26	13	10	5.0	1604	802	525	263
PD 115,934	109	0.26	160	0.38	31	0.07	374	0.88	340	0.80
PD 117,213	325	0.81	210	0.53	79	0.20	609	1.5	705	1.8
PD 117,469	33	3.3	76	7.6	12	1.2	3453	345	156	16
PD 117,756	45	25	117	65	20	11	1678	932	19	11
PD 117,802	91	9.3	90	9.2	24	2.4	1065	109	566	58
PD 119,556	670	146	877	191	443	96	3924	853	573	125
PD 121,436	18	46	7.8	20	51	131	3045	7808	294	754
Adriamycin	46	1.0	96	2.1	88	2.0	91	2.0	466	10
Mitoxantrone	23	25	7.7	8.4	17	13	144	157	164	178
Amsacrine	402	15	957	37	743	29	667	26	2811	108

^a Expressed in nM. Values represent the mean of at least two determinations.

^b IC₅₀ against the indicated cell line relative to the IC₅₀ against L1210.

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*Activity of the pyrazoloacridines in the
human tumor stem cell clonogenic assay*

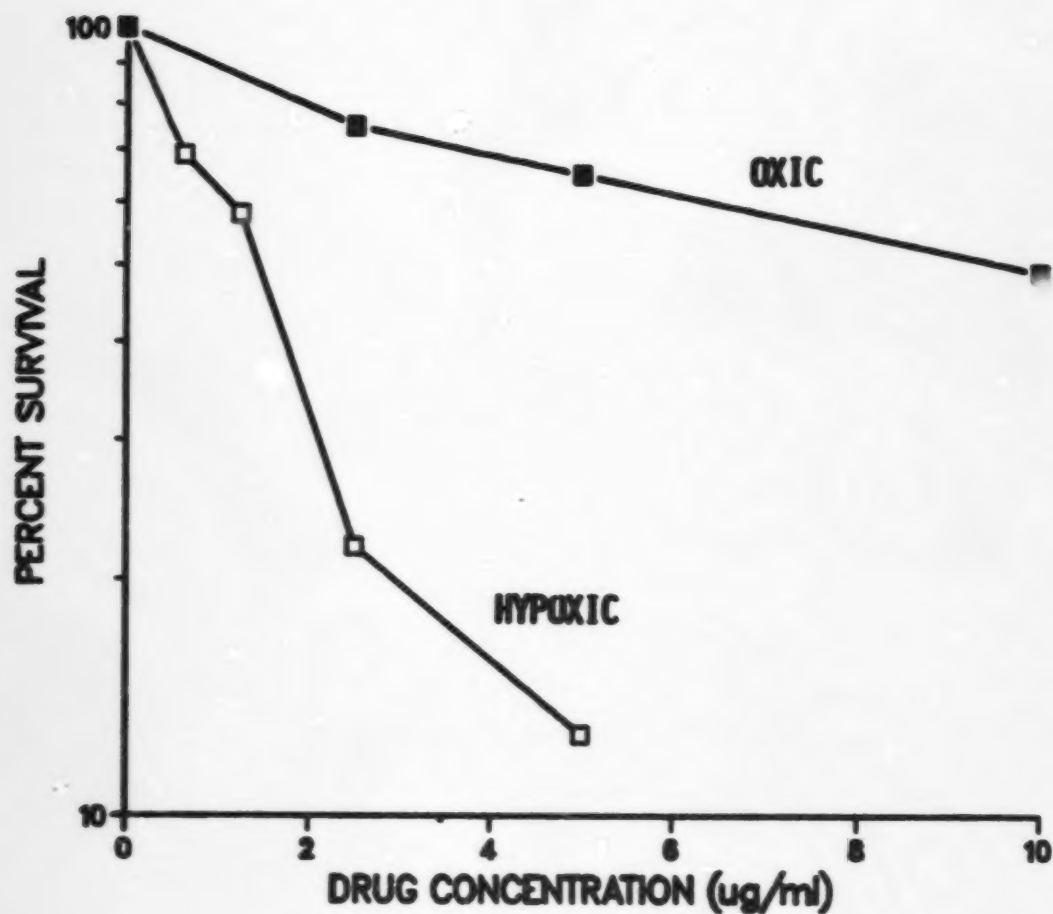
Tumor samples of either breast, lung, or ovarian origin were treated for one hour at the indicated drug concentrations prior to plating, as previously described (18). A positive response is defined as $\leq 50\%$ survival.

Compound	L1210 Potency ($\mu\text{g/ml}$) ^a	Concentration ($\mu\text{g/ml}$)	Sensitive/Total (%)
PD 111,245	1.37	1.0	6/28 (21)
		4.0	23/28 (82)
PD 114,541	0.002	0.0025	4/26 (15)
		0.01	5/26 (19)
PD 115,934	1.07	1.0	8/27 (30)
		4.0	24/27 (89)
PD 117,469	0.049	0.05	0/26 (0)
		0.2	5/26 (19)
PD 117,802	0.056	0.05	4/17 (24)
		0.2	6/16 (31)
Mitoxantrone	0.005	0.1	8/36 (22)
		0.4	27/36 (47)

^a IC₅₀ in a clonogenic assay after one hour treatment.

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CYTOTOXICITY OF PD 114,245 AGAINST OXIC AND HYPOXIC HCT-8 CELLS



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**Cytotoxicity of the pyrazoloacridines against cycling
and noncycling Chinese hamster ovary cells**

Cultures which were either exponentially growing or nondividing were treated for one hour prior to plating for clonogenic survival as described in "Materials and Methods." Data is expressed as the mean $\pm$ S.E. of at least three determinations.

Compound	IC ₅₀ (X 10 ⁻⁶ M)		Ratio ^a
	Exponential	Plateau	
PD 111,425	7.61 $\pm$ 0.16	19.3 $\pm$ 3.0	2.5
PD 113,377	37.7 $\pm$ 4.2	112 $\pm$ 1.1	3.0
PD 114,245	14.9 $\pm$ 4.0	64.6 $\pm$ 4.5	4.3
PD 114,541	0.047 $\pm$ 0.016	2.50 $\pm$ 0.61	53
PD 115,934	57.2 $\pm$ 1.1	125 $\pm$ 56	2.2
PD 117,469	0.16 $\pm$ 0.028	52.9 $\pm$ 11.1	331
PD 117,756	0.33 $\pm$ 0.15	16.2 $\pm$ 3.0	49
PD 117,802	0.12 $\pm$ 0.012	20.3 $\pm$ 4.1	169
PD 118,035	39.5 $\pm$ 15.8	60.0 $\pm$ 26.7	1.5
PD 119,556	0.29 $\pm$ 0.013	73.2 $\pm$ 20.6	252

Adriamycin	0.85 $\pm$ 0.20	32.2 $\pm$ 4.9	38
Ansacrine	0.11 $\pm$ 0.003	3.12 $\pm$ 0.17	28
Actinomycin D	0.65 $\pm$ 0.10	1.27 $\pm$ 0.17	2.0

^a IC₅₀ (Plateau)/IC₅₀ (Exponential)

*Inhibitory effects of the pyrazoloacridines against
DNA And RNA synthesis in L1210 cells*

Results are expressed as the micromolar concentration of drug required to inhibit [³H]thymidine or [³H]-uridine incorporation by 50 percent. Values represent the mean $\pm$ S.E. of at least three determinations.

Compound	DNA	RNA	Ratio ^a
PD 110,334	1.81 $\pm$ 0.39	2.30 $\pm$ 0.30	0.79
PD 111,425	1.81 $\pm$ 0.40	1.11 $\pm$ 0.23	1.63
PD 113,377	1.72 $\pm$ 0.12	1.39 $\pm$ 0.19	1.24
PD 114,245	1.99 $\pm$ 0.38	1.08 $\pm$ 0.13	1.84
PD 114,541	1.21 $\pm$ 0.34	0.76 $\pm$ 0.18	1.59
PD 115,934	6.19 $\pm$ 0.79	1.32 $\pm$ 0.43	4.69
PD 117,469	2.49 $\pm$ 0.56	3.89 $\pm$ 0.98	0.64
PD 117,756	0.32 $\pm$ 0.049	0.45 $\pm$ 0.13	0.71
PD 117,802	1.49 $\pm$ 0.51	1.45 $\pm$ 0.24	1.03
PD 119,556	0.25 $\pm$ 0.076	0.84 $\pm$ 0.083	0.30
Adriamycin	1.33 $\pm$ 0.33	1.24 $\pm$ 0.73	1.07
Amsacrine	1.11 $\pm$ 0.26	41.0 $\pm$ 5.0	0.03
Actinomycin D	0.49 $\pm$ 0.10	0.059 $\pm$ 0.026	8.31

^aIC₅₀ (DNA)/IC₅₀ (RNA)

IN VITRO ACTIVITY OF THE PYRAZOLOACRIDINES AGAINST
MULTIDRUG-RESISTANT CELL LINES

COMPOUND	P388	P388/ADR	(R/S)	B16			16c	16c/ADR	(R/S)
				B16/ADR	(R/S)	IC50 IN NM			
PD 110334	438	699	(1.6)	51	96	(1.9)	47	145	(3.1)
113377	1038	990	(1.0)	107	157	(1.5)	25	18	(0.7)
114245	432	465	(1.1)	174	212	(1.2)	19	42	(2.2)
114541	3.4	9.3	(2.7)	1.4	3.0	(2.1)	1.6	4.1	(2.6)
115934	1052	1153	(1.1)	224	306	(1.4)	45	39	(0.9)
117469	4.6	27	(5.9)	1.5	5.1	(3.4)	--	--	--
117802	5.6	54	(9.6)	3.7	6.7	(1.8)	1.8	6.4	(3.6)
119556	10	218	(22)	18	21	(1.2)	--	--	--

ADRIAMYCIN	64	8812	(138)	23	393	(17)	17	121	(7.1)
AMSACRINE	29	907	(31)	41	73	(1.8)	27	125	(4.6)
MITOXANTRONE	6.3	374	(59)	4.1	16	(3.9)	--	--	--

R/S = IC₅₀ (RESISTANT)/IC₅₀ (SENSITIVE)

GROWTH INHIBITION WAS ASSESSED AFTER 3-4 DAYS OF CONTINUOUS EXPOSURE TO DRUG.

ACTIVITY OF THE PYRAZOLOACRIDINES AGAINST
 ADRIAMYCIN-RESISTANT B16 MELANOMA

<u>AGENT</u>	<u>B16</u>		<u>B16/ADR</u>	
	<u>NET KILL</u>	<u>% T/C</u>	<u>NET KILL</u>	<u>% T/C</u>
ADRIAMYCIN	1.3	223	0.0	144
	1.0	192	-0.5	126
PD 114541	1.0	208	1.1	179
	0.2	165	1.6	199
PD 115934	0.4	179	1.8	204
	-1.0	122	1.2	185

IP/IP

IN VIVO ACTIVITY OF THE PYRAZOLOACRIDINES

	COMPOUND				
	<u>PD 114541</u>	<u>115934</u>	<u>117469</u>	<u>118035</u>	<u>119556</u>
	% T/C (# CURES)				
L1210 [IP/IP; D1-5]	352 (3/6)	225 (2/6)	332 (4/6)	230 (1/6)	361 (3/6)
P388 [IP/IP; D1-5]	180	261 (2/6)	263 (5/6)	275 (2/6)	275 (6/6)
B16 [IP/IP; D1-9]	158	135	217	134	256 (8/10)
M5076 [IP/IP; D1,5,9,13]	239 (6/6)	170	298 (8/10)	201	186
MX-1 [SR/IP; D1,5,9]	7	13	21	28	-53 (3/6)
LOX [IP/IP; D1,5,9]	157	126	213	115	340 (3/6)
A549 [SR/IP; D1,5,9]	---	14	---	---	0
ROS [SC/SC; D3,7,11]	4.0	2.0	0	28	55
[SC/IV; Q4HX3; D3,7,11]	10	26	0	6.0	19
	GROWTH DELAY				
16C [SC/SC; D1,5,9]	11.6	14.2	10.1	10.1	3.5
[SC/IV; Q4HX3; D1,5,9]	13.9	12.9	12.8	14.0	8.4
C11A [SC/SC; D1,5,9]	5.8	17.1	7.3	9.2	2.9
[SC/IV; Q4HX3; D1,5,9]	8.9	13.5	12.2	12.7	10.8
HCT-8 [SC/SC; D7,11,15]	6.4	4.3	---	---	6.3

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FACTORS THAT DEFEAT CANCER CHEMOTHERAPY

- 1. HOST TOXICITY.**
- 2. UNFAVORABLE KINETICS OF ADVANCED, HIGHLY METASTATIC TUMORS.**
- 3. INHERENT DRUG RESISTANCE OF CERTAIN TUMORS SUCH AS THAT OBSERVED FOR COLORECTAL CANCER.**
- 4. ACQUIRED DRUG RESISTANCE:**
 - A. DRUG-SPECIFIC RESISTANCE.**
 - B. MULTIDRUG RESISTANCE (PLEIOTROPIC DRUG RESISTANCE).**

THE PROBLEM OF DRUG RESISTANCE IS ENCOUNTERED IN TWO DISTINCT CLINICAL SETTINGS AND, IN EACH, IT HAS DEVASTATING CONSEQUENCES FOR THE PATIENT.

- 1. THE FAILURE OF INITIAL CHEMOTHERAPY.**
- 2. DEVELOPMENT OF RESISTANCE TO CHEMOTHERAPY IN PATIENTS WHO HAVE INITIALLY RESPONDED BUT HAVE THEN RELAPSED. THIS PROBLEM IS FURTHER EXACERBATED BY THE OBSERVATION THAT THESE TUMORS ARE OFTEN CROSS-RESISTANT TO OTHER DRUGS EVEN THOUGH THESE DRUGS WERE NOT USED IN THE INITIAL TREATMENT.**

GOALS

- 1. TO DEVELOP STRATEGIES OR IDENTIFY AGENTS THAT WILL REVERSE THE DRUG RESISTANCE OF MULTIDRUG RESISTANT CELLS TOWARD OUR PRESENT LEAD ANTICANCER COMPOUNDS.**
- 2. TO DEVELOP NEW ANTICANCER DRUGS THAT ARE ACTIVE AGAINST MULTIDRUG RESISTANT TUMORS.**
- 3. TO DEVELOP NEW ANTICANCER DRUGS THAT ARE ACTIVE AGAINST THE INTRINSICALLY DRUG RESISTANT COLON TUMORS.**

P388/ADR MULTIDRUG RESISTANT PHENOTYPE

- 1. P388/ADR CELLS ARE CROSS-RESISTANT TO STRUCTURALLY AND FUNCTIONALLY DISTINCT COMPOUNDS.**
- 2. CALCIUM CHANNEL BLOCKERS SUCH AS VERAPAMIL AND CALMODULIN ANTAGONISTS SUCH AS TRIFLUOPERAZINE CAN PARTIALLY REVERSE THE DRUG RESISTANCE OF P388/ADR CELLS.**
- 3. P388/ADR CELLS HAVE AN INCREASED EXPRESSION OF P GLYCOPROTEIN.**
- 4. REDUCED DRUG ACCUMULATION IN P388/ADR CELLS IS DUE IN PART TO AN ACTIVE ENERGY DEPENDENT EFFLUX MECHANISM.**
- 5. P388/ADR CELLS ALSO HAVE REDUCED DRUG INFLUX.**
- 6. REDUCTION IN ADRIAMYCIN-INDUCED DNA DAMAGE.**

TABLE 1
 SENSITIVITY OF CULTURED P388S AND P388R LEUKEMIA CELLS
 TO ANTITUMOR AGENTS

COMPOUND	ID ₅₀ (nM) ^A		DEGREE OF RESISTANCE ^B
	P388S	P388R	
DAUNOMYCIN	110.0 ± 4.0	4151.7 ± 555.5	38
ADRIAMYCIN	128.8 ± 9.6	6586.5 ± 662.3	51
MARCELLOMYCIN	14.5 ± 3.4	543.0 ± 9.7	37
VINBLASTINE	3.7 ± 1.4	161.5 ± 5.7	44
M-AMSA	50.9 ± 0.8	851.4 ± 12.3	17
VP-16	21.0 ± 3.1	5760.0 ± 401.4	274
VN-26	3.3 ± 1.1	1258.2 ± 81.6	381
TRIMETREXATE	12.2 ± 3.2	269.5 ± 2.7	22
CI-937	157.0 ± 9.8	46781.0 ± 1282	298
CI-942	498.0 ± 23.1	75870.0 ± 5089	152
CI-921	84.0 ± 2.4	357.3 ± 10.8	4.3
CI-958	668.3 ± 59.1	3074.0 ± 311.5	4.6

^ACELLS WERE INCUBATED FOR 72 HOURS WITH THE INDICATED DRUG. RESULTS ARE THE MEAN OF THREE EXPERIMENTS, EACH PERFORMED IN DUPLICATE ± S.E.

^BDEGREE OF RESISTANCE = $\frac{\text{ID}_{50} \text{ OF P388R}}{\text{ID}_{50} \text{ OF P388S}}$

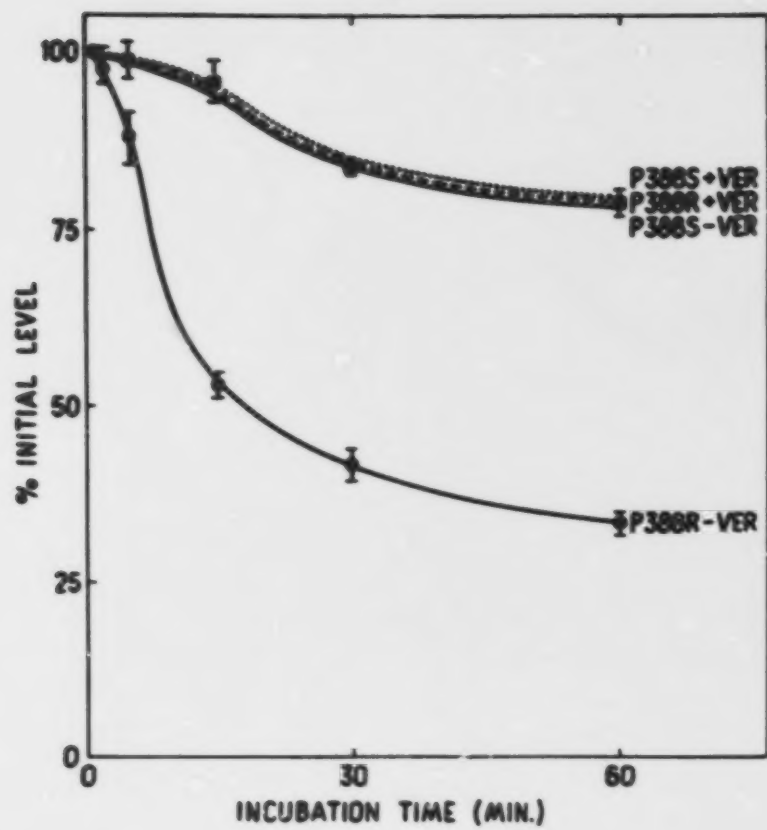
Potentiation by verapamil of anthracycline and anthrapyrazole activity in pleiotropically-resistant P388 leukemia cells

Compound	ID ₅₀ (nM) ^a				Degree of Resistance	Degree of Potentiation ^b
	P388S		P388R			
	Verapamil - +		Verapamil - +			
PD 111,391	163	178	463	328	2.8	1.4
PD 110,911	127	114	628	437	4.9	1.4
PD 112,940	141	145	3227	1014	23	3.2
CI-942 —	498	468	75870	4069	152	18
CI-937	157	161	46781	1263	298	37
Adriamycin	129	121	6587	626	51	10.5
Daunomycin	110	121	4152	464	38	9.0

^a Cells were grown for 72 hours in the presence of the indicated drug + 5 ug/ml verapamil. Results are the mean of 2 experiments each performed in triplicate.

^b Degree of potentiation = $\frac{\text{ID}_{50} \text{ of P388R minus verapamil}}{\text{ID}_{50} \text{ of P388R plus verapamil}}$

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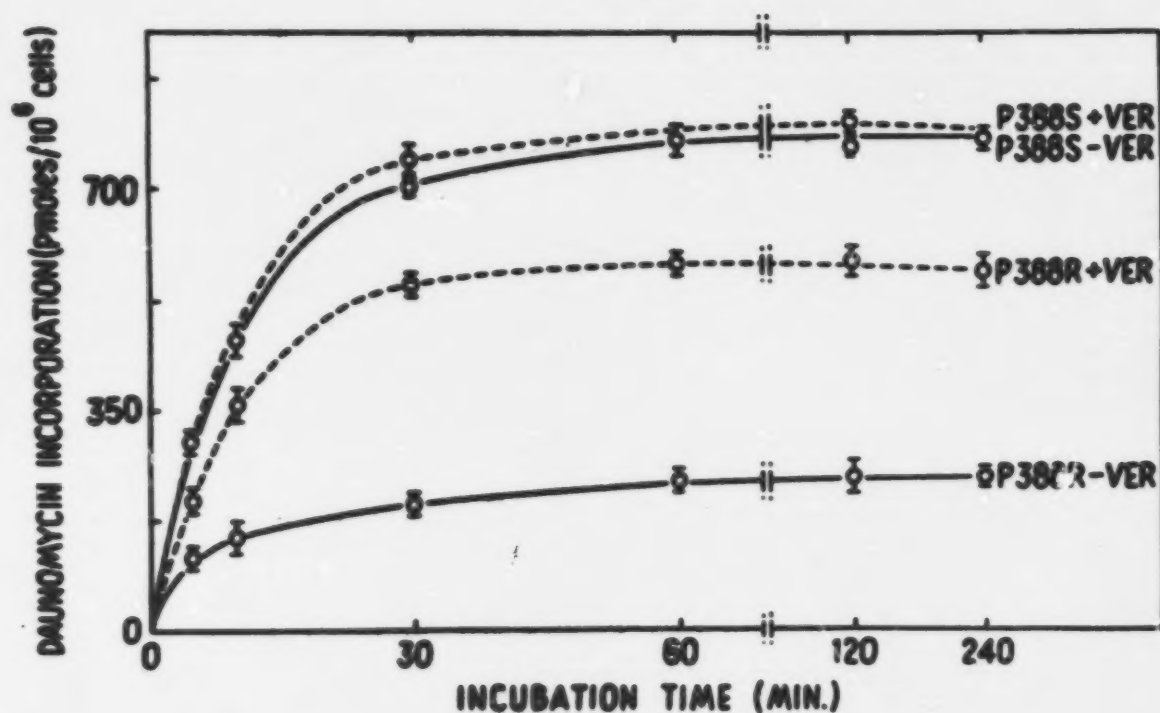
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**Influx and Efflux of Daunomycin in
P388S and P388R Cells**

Cell Type	+/- Verapamil (10 ⁻⁶ ug/ml)	Influx ¹ (pmoles daunomycin/min/10 ⁶ cells)	Efflux ¹
P388S	-	45.5 ± 4.1	2.40 ± 0.31
	+	43.9 ± 4.8	2.15 ± 0.23
P388R	-	28.0 ± 1.9	16.5 ± 1.5
	+	26.6 ± 2.2	1.96 ± 0.27

¹ Influx of [³H]daunomycin was measured over the first minute. Incubations were terminated by rapid chilling and centrifugation. For efflux experiments, cells were first incubated with daunomycin for 10 minutes after which outward transport was measured over the initial minute of efflux as described by Kessel and Wheeler (9). Influx and efflux were determined by linear regression analysis. Results are the mean of three experiments, each performed in duplicate ± S.E.

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VERAPAMIL SHORTCOMINGS:

1. VERAPAMIL DOES NOT COMPLETELY REVERSE DRUG RESISTANCE IN P388/ADR CELLS.
2. CLINICALLY, TOLERABLE LEVELS OF VERAPAMIL ARE TOO LOW TO INHIBIT DRUG EFFLUX.

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SUMMARY OF THE EVALUATION OF POTENTIAL POTENTIATORS OF ADRIAMYCIN, TRIMETREXATE AND
CI-921 IN P388/R CELLS

COMPOUND	CONCENTRATION (UG/ML)	DEGREE OF POTENTIATION IN P388/R FOR:		
		ADR	TMQ	CI-921
VERAPAMIL	5.0	10.5	9.2	3.0
DILTIAZEM	5.0	3.2		
NIFEDIPINE	1.0	1.2		
NITRONIFENE	2.5	3.3		
AMIODARONE	5.0	33.5	13.7	4.5
TIAPAMIL	1.0	1.0		
TIAPAMIL-2933	1.0	27	15.7	3.2
QUINIDINE	5.0	7.0		
RESERPINE	5.0	20.3	38.0	3.0
TRIFLUOPERIZINE	1.0	2.2		
COMPazine	1.0	2.2		
MELITTIN	10.0	6.8		
CALMIDAZOLIUM	15.0	2.3		
H-7	1.0	1.2		
H-7	10.0	5.3		
H-9	10.0	5.9		
PD 405,678	5.0	2.0		
RENADRYL	25.0	11.8		
CHLOROPHENIRAMINE	50.0	4.6		
QUINACRINE	2.5	9.4		
TRIPARANOL	5.0	5.9		
TWEEN 80	25	9.9		
TRITON WR-1339	25	11.8		

THE DEGREE OF RESISTANCE OF P388/R TO:

ADRIAMYCIN (ADR) = 51
TRIMETREXATE (TMQ) = 22
CI-921 = 4.2

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RELATIONSHIP BETWEEN AMIODARONE DOSAGE
AND APPARENT STEADY-STATE SERUM CONCENTRATIONS¹

	DAILY AMIODARONE DOSE		
	200 MG	400 MG	600 MG
PATIENTS, N	41	75	36
DOSE, MG/KG BODY WEIGHT	2.8 ± 0.5	5.4 ± 0.9	7.6 ± 1.6
AMIODARONE CONCENTRATION µG/ML	1.2 ± 0.5	2.5 ± 1.1	3.7 ± 1.4

¹FROM ROTHENSCH ET AL., ANNALS OF INT. MED. 101: 462-469, 1984.

CONCLUSION FOR GOAL #1:

THE COMBINATION OF AMIODARONE AND CI-921 OR RESERPINE
AND TRIMETREXATE APPEARS PROMISING IN OVERCOMING
DRUG RESISTANCE IN MULTIDRUG RESISTANT TUMORS.

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**RATIONALE FOR THE DEVELOPMENT OF
LIPOPHILIC ANTIFOLATES ACTIVE AGAINST
MULTIDRUG RESISTANT TUMORS**

1. LIPOPHILIC

- A. TIAPAMIL-2933 > VERAPAMIL > TIAPAMIL.**
- B. ANTHRAPHYRAZOLES.**
- C. PYRAZOLOACRIDINES.**
- D. METOPRINE > TRIMETREXATE.**

2. AMPHIPATHIC.

3. PK.

4. REDUCTION IN ADRIAMYCIN-INDUCED DNA DAMAGE.

**THE KEY TO DEVELOPING NEW ANTICANCER DRUGS THAT
ARE AS ACTIVE IN MULTIDRUG RESISTANT TUMORS AS
THEY ARE IN DRUG SENSITIVE TUMORS SHOULD BE RELATED
IN PART TO THE ABILITY OF THESE DRUGS TO AVOID
THE ACTIVE EFFLUX MECHANISM EITHER BY INHIBITING
OR CIRCUMVENTING IT.**

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COMPARISON OF THE LIPOPHILICITY OF THE
CALCIUM CHANNEL BLOCKERS VERAPAMIL, TIAPAMIL
AND THE TIAPAMIL ANALOG, 2933

<u>Compound</u>	<u>Lipophilicity</u>	
	<u>Chloroform/Water</u>	<u>Octanol/Water</u>
Verapamil	52.19	1.66
Tiapamil	20.83	0.28
2933	68.75	3.26

Relationship Of The Lipophilic And DNA Binding Properties
of the Anthrapyrazoles With Their Effect On P388 Cells

<u>Anthrapyrazole</u>	<u>Degree of Resistance</u>	<u>Partition Ratio¹</u>		<u>DNA Ethidium Displacement C₅₀ (nM)²</u>
		<u>Chloroform/Water</u>	<u>Octanol/Water</u>	
PD 111,391	2.8	0.79 $\pm$ 0.06	2.57 $\pm$ 0.17	142 $\pm$ 8.75
PD 110,911	5.0	0.15 $\pm$ 0.02	0.33 $\pm$ 0.02	30 $\pm$ 1.00
PD 112,940	23	0.033 $\pm$ 0.001	0.14 $\pm$ 0.01	27 $\pm$ 1.47
CI-942	152	0.030 $\pm$ 0.004	0.13 $\pm$ 0.01	33 $\pm$ 0.75
CI-937	297	0.070 $\pm$ 0.004	0.13 $\pm$ 0.01	25 $\pm$ 1.70

¹ Partition ratios were performed with 10 ug/ml of drug at the emission maximum following procedures described by Skovsgaard (31). Results are the mean of two experiments, each performed in triplicate $\pm$ S.E.

² DNA ethidium displacement assays were performed as described by Baguley et al (32). C₅₀ = the concentration of drug required to displace 50% of DNA-bound ethidium. Results are mean of two experiments, each performed in triplicate $\pm$ S.E.

**EFFECT OF LIPOPHILIC ANTIFOLATES ON
MULTIDRUG RESISTANT P388 LEUKEMIA CELLS**

<u>ANTIFOLATES</u>	<u>IC50 (NG/ML)</u>		<u>DEGREE OF RESISTANCE</u>
	<u>P388S</u>	<u>P388/ADR</u>	
TRIMETREXATE	6.9	152	22
PD 82,216	21	28	1.3
PD 80,198	3.9	3.9	1.0
PD 81,132	12	15	1.3
PD 75,825	5.7	5.7	1.0
PD 73,666	12.9	8.1	0.63

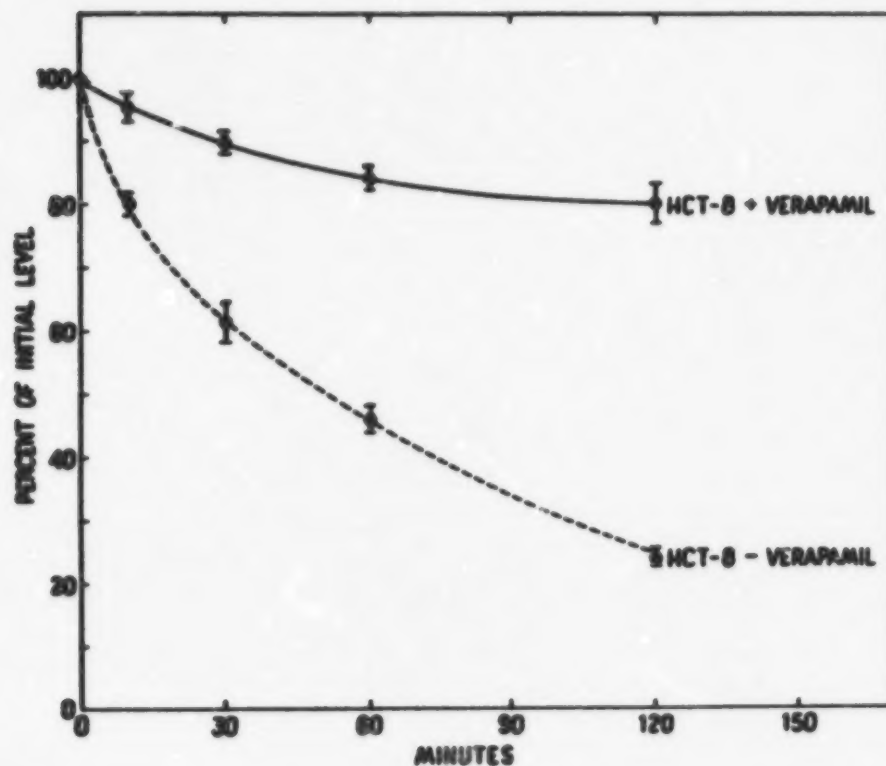
CONCLUSION FOR GOAL #2:

**CERTAIN LIPOPHILIC ANTIFOLATES RETAIN FULL ACTIVITY
AGAINST MULTIDRUG RESISTANT P388 LEUKEMIA WHEN
COMPARED TO THEIR DRUG-SENSITIVE PARENT CELL LINE.**

**POSSIBLE MECHANISMS OF DRUG RESISTANCE
IN COLON CANCER**

1. REDUCED RADIATION AND DRUG-INDUCED DNA DAMAGE.
2. ALTERED DNA REPAIR ENZYMES (?).
3. LOWER SUSCEPTIBILITY TO FREE RADICAL-INDUCED DAMAGE.
4. ELEVATED LEVELS OF GSH AND METALLOTHIONEIN (?).
5. LOW AFFINITY MTX CARRIER AND LOW NET POLY-GLUTAMYLATION OF MTX.
6. NONSPECIFIC, ENERGY DEPENDENT DRUG EFFLUX MECHANISM WHICH CAN BE INHIBITED BY VER.

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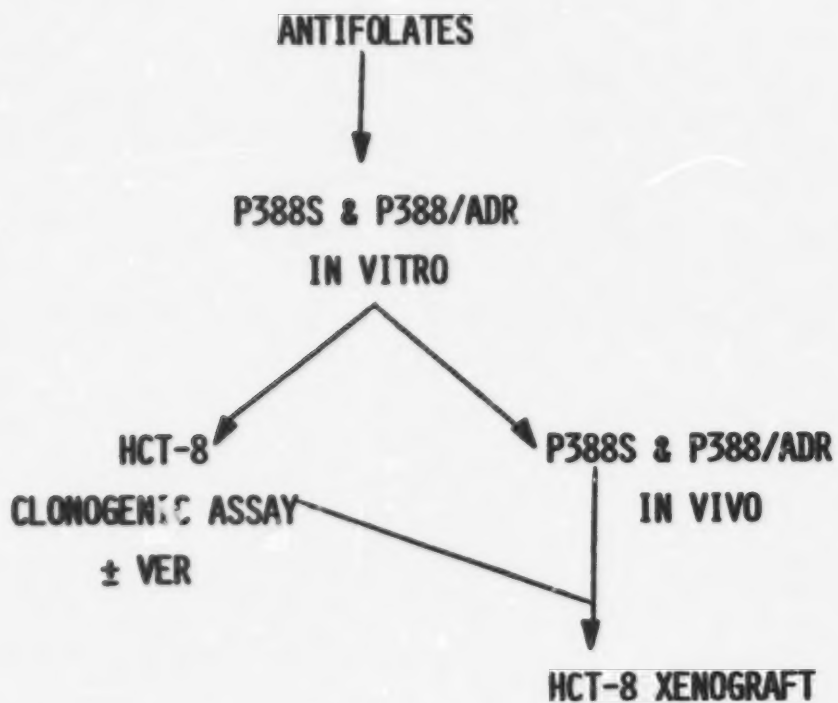


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**DETECTION OF DRUG EFFLUX IN COLON
TUMOR CELL LINES**

CELL LINE	DRUG (ADR) EFFLUX +/-
COLON-38	-
WiDr	-
HCT-8	+
HT-29	+
COLON-26	+
LoVo	+
LS-174T	+

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CONCLUSION:

OUR STUDIES ON COLON TUMOR CELL LINES HAVE LED TO THE DISCOVERY THAT THE INTRINSIC DRUG RESISTANCE OF COLON TUMORS INVOLVES, AT LEAST IN PART, A NONSPECIFIC ENERGY DEPENDENT DRUG EFFLUX MECHANISM WHICH IS SIMILAR TO THE DRUG EFFLUX MECHANISM IDENTIFIED IN MULTIDRUG RESISTANT TUMORS. WE HAVE DEMONSTRATED THAT CERTAIN LIPOPHILIC ANTIFOLATES CAN SOMEHOW BYPASS OR INHIBIT THIS EFFLUX PROCESS AND ARE, THEREFORE, AS ACTIVE IN MULTIDRUG CELLS AS THEY ARE IN DRUG SENSITIVE CELLS. BASED ON OUR FINDINGS FOR DRUG EFFLUX IN COLON TUMOR CELLS, WE EXPECT THAT THESE SAME ANTIFOLATES WOULD ALSO BE EFFECTIVE AGAINST COLON TUMORS.

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NEW SYNTHETIC ANTICANCER LEADS

- Program strategies
- Medicinal chemistry approaches
 - Preselection
 - Computer models
 - Structure-function analysis
 - Congener/pro-drug synthesis
- Examples of current leads
- Compound input to the new "disease-oriented" screens
- Future directions

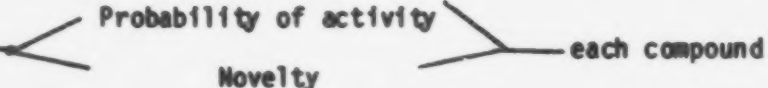
PRESELECTION APPROACHES

- Biological criteria
- Chemical criteria
- Computer model

COMPUTER MODEL

Basis: Molecular structural features as predictors of biological activity

Methodology:

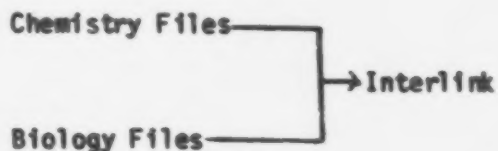
- Utilizes a "training set" - collection of compounds with known structural and biological activity
- Computer programs - uses and manipulates data from a "training set" for a given system
- Assigns ———  each compound

STRUCTURE - ACTIVITY ANALYSIS

OBJECTIVES:

- To Identify Gap Areas for Acquisition/Synthesis
- To Deemphasize Over-Worked Areas

METHODOLOGY:



LEAD OPTIMIZATION

GOALS:

- Bioavailability
- Stability
- Potency
- Specificity

METHODS

CHANGE SUBSTITUTIONS TO ALTER:

- Chemical Reactivity
- Electron Density
- Log P, Pka
- Molecular size & shape

PRECLINICAL SCREENING SUCCESSES

COMPOUND	CURATIVE ANIMAL TUMOR MODEL(S)
Tiazofurin	Lewis Lung
Herbarone	L1210 Leukemia
Flavone Acetic Acid	Colon 38
Oxanthrazole	L1210 Leukemia B16 Melanoma Mx-1 Mammary Xenograft and M5076 Sarcoma

FUTURE DIRECTIONS

Basis: Emerging data from the new "disease-oriented" screens

Approaches:

- Lead-optimization through congener/pro-drug synthesis
- Structure-function analysis
- Computer model using "training sets" for each of the new screens

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PHASE I WORKING GROUP

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Amonafide

Patient Characteristics

<u>Characteristic</u>	<u>No.</u>
Males/Females	19/3
Median age (Range)	57 yrs (42-73 yrs)
Performance Status (SWOG)	
0	3
1	12
2	9
3	3
Prior Therapy	
Chemotherapy	20
Radiotherapy	15
None	3

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Amonafide

Patient Characteristics

Disease Types

<u>Diagnosis</u>	<u>No. Patients</u>
Lung	8
Colorectal	5
Renal Cell	3
Prostate	3
Endometrium	2
Head and Neck	2
Pancreas	2

Amonafide
T85-0188
UTHSC
November 1986

University of Texas - San Antonio

Amonafide

Dosage Escalation

<u>Dosage Level (mg/m²)</u>	<u>No. Patients</u>	<u>No. Courses</u>
18	1	1
220	3	8
293	3	10
390	3	9
519	4	12
690	6	10
800	6	7
918	<u>1</u>	<u>1</u>
	27	58

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Non-hematological Toxicity

Nausea/Vomiting

	<u>Dose level (mg/m²)</u>							
<u>Grade</u>	<u>18</u>	<u>220</u>	<u>293</u>	<u>390</u>	<u>519</u>	<u>690</u>	<u>800</u>	<u>918</u>
1	0	1	2	0	4	3	4	1
2	0	0	1	0	1	1	0	0
<u>Total courses at level (n = 58)</u>	1	8	10	9	12	10	7	1

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Non-Hematological Toxicity

Other

<u>Toxicity</u>	<u>#Courses (dose level)</u>
Diaphoresis/Flushing	7 (293, 519, 690, 800, 918)
Dizziness	4 (519, 690, 918)
Alteration of Taste	3 (220, 390, 800)
Anorexia	4 (293, 519, 690)
Other: Headache (2), tinnitus (3), weakness/malaise (2), circumoral numbness (1), urticaria (1)	

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Hematological Toxicity

Leukopenia

<u>Grade</u>	<u>Dose level (mg/m²)</u>						
	<u>18</u>	<u>220</u>	<u>293</u>	<u>390</u>	<u>519</u>	<u>690</u>	<u>800</u> <u>918</u>
1		1 (3.1)					
2						2 (2.2, 2.6)	
3							
4							1* 1** (0.9) (0.3)

* Thrombocytopenia (24,000)

** Death from sepsis

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Antitumor Activity

Pt #10 - Lung Carcinoma - CR at 390 mg/m²

**Pt #11 - Prostate Carcinoma - Excellent
subjective response at 519 mg/m²**

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Summary

- (1) MTD = 800mg/m² IV bolus Q 28 days
- (2) Recommended dosage for phase II trials = 690 mg/m²
- (3) DLT: myelosuppression (leukopenia)
- (4) Other toxicities: flushing/diaphoresis, nausea/vomiting, anorexia, dizziness, alteration of taste
- (5) Antitumor activity: NSCLC, ? Prostate

BENZISOQUINOLINEDIONE

METHODS

TWO SCHEDULES WERE INVESTIGATED: A SINGLE INFUSION AND A DAILY INFUSION TIMES FIVE, EACH REPEATED EVERY 21 DAYS. INFUSIONS WERE GIVEN OVER 10 TO 30 MINUTES INITIALLY BUT WERE PROLONGED TO ONE HOUR WHEN ACUTE TOXICITIES DEVELOPED THAT SEEMED RELATED TO THE RATE OF INFUSION. EACH SCHEDULE STARTED AT 1/10 MELD 10 AND ESCALATED ACCORDING TO THE MODIFIED FIBONACCI SEARCH SCHEME.

BENZISOQUINOLINEDIONE PATIENT CHARACTERISTICS

NUMBER OF PATIENTS (M/F)	66 (41/25)
MEDIAN AGE (RANGE)	59.5(22-77)
PERFORMANCE STATUS: 0	10 PATIENTS
1	11
2	19
3	25
4	1

BENZISOQUINOLINEDIONE

PATIENT DIAGNOSES

COLORECTAL CARCINOMA	19
RENAL CARCINOMA	10
ADENOCARCINOMA, UNKNOWN PRIMARY	5
OVARIAN CARCINOMA	4
SMALL CELL LUNG CARCINOMA	4
HEAD AND NECK CARCINOMA, SQUAMOUS	3
ADENOIDCYSTIC CARCINOMA	3
SOFT TISSUE SARCOMA	3
NEUROBLASTOMA	2
BREAST CARCINOMA	2
BLADDER CARCINOMA	2
NON-SMALL CELL LUNG CARCINOMA	2

* SINGLE PATIENTS HAD HEPATOCELLULAR, CLOACOGENIC, PANCREATIC AND PROSTATE CANCERS, SQUAMOUS CARCINOMAS OF THE SCROTUM AND THE ESOPHAGUS, AND ADENOCARCINOMA OF THE DUODENUM.

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BENZISOQUINOLINEDIONE, DAILY X 5

NONHEMATOLOGIC TOXICITY

EPISODES OF TOXICITY (MEDIAN GRADE)

DOSE (MG/M ² /D)	NUMBER OF EVALUABLE COURSES	GI	WARMTH, FLUSHING	DIZZINESS	HEADACHE	EARS RINGING	LOCAL REACTION
7.6	5	--	--	--	--	--	--
15.2	5	1(1)	--	--	--	--	--
25.1	5	--	--	--	--	--	--
38.0	9	2(1)	--	--	--	--	--
53.2	3	--	--	--	--	--	--
68.4	3	1(1)	1(1)	--	--	--	--
91.2	4	1(1)	1(1)	1(2)	--	--	--
121.6	7	3(1)	4(1)	3(1)	--	--	--
*162.0	3	1(1)	2(1)	--	--	--	--
*216.2	6	3(1)	3(1)	--	--	--	--
*288.2	8	5(1)	2(1)	2(1)	1(1)	1(1)	3(2)
*350.0	3	1(1)	1(1)	--	1(1)	1(1)	--

*Infusion lengthened to one hour

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BENZISOQUINOLINEDIONE

HEMATOLOGIC TOXICITY - SINGLE BOLUS

DOSE (NG/M ²)	NUMBER OF PATIENTS	NUMBER OF EVALUABLE COURSES	WBC NADIR * MEDIAN (RANGE)	PLATELET NADIR* MEDIAN (RANGE)
18.4	3	3	3.7(3.6-7.6)	140(99-272)
36.8	4	6	9.1(5.3-13.3)	276(248-342)
60.7	5	7	8.6(3.1-12.8)	243(123-404)
92.0	7	8	5.0(4.0-8.9)	268(196-487)
128.8	4	4	7.9(3.3-11.8)	396(108-484)
165.6	4	4	5.8(2.7-6.7)	207(73-436)
220.8	4	10	6.5(4.4-8.7)	336(127-509)
294.4	5	6	4.7(3.7-24.8)	151(48-312)
392.5	3	4	17.7(6.0-30.8)	433(217-501)
523.4	8	18	3.4(1.5-9.3)	182(47-457)
697.8	3	5	4.9(3.9-5.5)	332(245-455)
800.0	4	2	4.3, 4.8	129, 158

*WBC and platelet count x 10³/mm³

BENZISOQUINOLINEDIONE, SINGLE BOLUS

NONHEMATOLOGIC TOXICITY

EPISODES OF TOXICITY (MEDIAN GRADE)

DOSE (MG/M ²)	NUMBER OF EVALUABLE COURSES	GI	WARMTH, FLUSHING	DIZZINESS	HEADACHE	EARS RINGING	SHORTNESS OF BREATH
18.4	3	1(1)	--	--	--	--	--
36.8	6	--	--	--	--	--	--
60.7	7	2(1)	--	--	--	--	--
92.0	9	--	--	--	--	--	--
128.8	4	1(1)	--	--	--	--	--
165.6	4	--	2(1)	--	--	--	--
220.8	10	2(1)	3(2)	5(1)	--	1(1)	1(2)
294.4	6	4(2)	4(1)	--	--	--	--
392.5	4	3(1)	3(1)	1(1)	--	--	--
*523.4	2	2(2)	2(2)	2(2)	--	1(2)	2(3)
*523.4	18	13(2)	6(1)	4(1)	1(1)	--	--
*697.8	5	3(1)	3(1)	1(1)	--	--	--
*800.0	5	5(2)	5(1)	1(1)	4(1)	--	--

* Infusion lengthened to one hour

* Infusion lengthened to two hours

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BENZISOQUINOLINEDIONE
HEMATOLOGIC TOXICITY - DAILY TIMES FIVE

DOSE MG/M ² /D	NUMBER OF PATIENTS	NUMBER OF EVALUABLE COURSES	WBC NADIR* MEDIAN(RANGE)	PLATELET NADIR* MEDIAN(RANGE)
7.6	3	5	5.9(3.1-7.9)	254(125-304)
15.2	4	5	8.8(5.3-16.5)	291(233-427)
25.1	4	5	9.3(3.1-25.0)	215(73-407)
38.0	5	9	4.8(4.2-15.6)	304(183-595)
53.2	3	3	7.9(2.6-9.6)	175(91-509)
68.4	4	3	6.7(5.5-6.7)	303(127-398)
91.2	4	4	7.2(3.0-7.8)	185(145-409)
121.6	5	7	6.7(3.4-10.7)	318(80-378)
162.5	3	3	5.3(3.2-5.7)	213(114-246)
216.2	3	6	5.7(2.9-10.2)	263(133-342)
288.2	6	8	2.8(0.6-5.1)	189(49-279)
350.0	3	2	0.7, 3.6	60, 227

* WBC and platelet count x 10³/ml

BENZISOQUINOLINEDIONE

CONCLUSIONS

1. BOTH SCHEDULES ARE NEARING THE MTD. IT APPEARS THAT THE DOSE LIMITING TOXICITY ON BOTH SCHEDULES WILL BE MYELO-SUPPRESSION.
2. SEVERE TOXICITY AT 523.4 MG/M² GIVEN OVER 20 MIN CONSISTING OF ACUTE ONSET VOMITING, MODERATE FLUSHING, DIZZINESS, TINNITUS, AND SHORTNESS OF BREATH WAS AMELIORATED BY LENGTHENING THE INFUSION TO ONE HOUR.
3. TOXICITY OTHER THAN MYELOSUPPRESSION HAS BEEN MILD TO MODERATE.
4. ONE PATIENT SUFFERED EXTRAVASATION OF DRUG WITH NO LONG TERM EFFECT.

AMONAFIDE (NAFIDIMIDE)

Pharmacology Report

THE OHIO STATE UNIVERSITY

A. INTRODUCTION

Clinical pharmacokinetic studies have been conducted in conjunction with the phase I evaluation of amonafide (NSC 308847, nafidimide, BIDA). Our present report summarizes the plasma pharmacokinetics in 32 patients following a single intravenous dose administered as a short infusion. Previously, we reported the plasma pharmacokinetics in 12 patients on the daily x 5 schedule in which the drug is given as a short infusion daily for 5 days and the urinary excretion of the parent drug in 26 of the patients on the single schedule. In addition, a summary of our studies to characterize the metabolites of amonafide is shown.

B. METHODS

1. The analytical method employed for the quantitation of amonafide in plasma and urine was described in the Phase I Working Group Minutes of November, 1985 together with typical calibration curves.

2. As the doses were escalated from 92.0 to 392.5 mg/m² on the single schedule, the duration of the infusion of the drug was increased from 10 to 20 minutes and finally to 30 minutes. At 523.4 mg/m², side effects including flushing, dizziness, wheezing, and shortness of breath occurred in two patients, which appeared to be related to the rate of drug administration. Accordingly, the duration of the infusion was increased and the pharmacokinetics at the 523.4 mg/m² level are reported for patients in which the drug was infused for one hour to 3 patients and 2 hours to another.

Patients treated with 697.8 mg/m² infused during one hour experienced no serious acute toxicity. Upon escalation of the dose to 930.4 mg/m² administered as a one hour infusion, extremely severe similar symptoms occurred in the first patient treated requiring discontinuing the infusion at about midway. Two drug related deaths occurred at approximately this dose level at another institution. Accordingly, the protocol was amended to decrease the dose to 800.0 mg/m² and extend the duration of the infusion to three hours. Five patients were entered at the latter dose level without acute episode.

3. Plasma concentration-time data for each patient study on the single schedule was fitted by the non-linear least squares computer program NONLIN to a three compartment open model with first-order elimination from the central compartment using the equations for a short intravenous infusion.

4. The isolation of amonafide and its basic metabolites and the procedure for isolation of the acidic metabolite from urine were summarized in the November, 1985 minutes.

C. RESULTS AND DISCUSSION

1. Amonafide is eliminated by a first-order process and plasma levels exhibit tri-exponential decay. Plasma concentration-time profiles determined at doses of 697.8 and 800.0 mg/m² are shown in Figs. 1 and 2. The plasma levels declined approximately 1000 fold during 72 hr after dosing. The curve in the plasma profiles drawn through the experimentally determined concentrations represent the computer fit to the data.
2. For 32 patients on the single dose schedule for the dose range 92.0-800.0 mg/m², the amonafide plasma profile is characterized by a short initial disposition phase ($t_{1/2\alpha} = 20.7$ min) followed by a beta phase ($t_{1/2\beta} = 2.69$ hr) and a prolonged terminal half-life ($t_{1/2\gamma} = 12.33$ hr) (Table 1). In these patients, the terminal half-lives ranged from 5.2 to 28.3 hr.
3. The mean residence time of the drug in the body (Vd_{ss}/Cl_T) was 6.91 hr with a range of 3.9 to 13.2 hr (Table 1).
4. The mean total body plasma clearance for patients on the single schedule was 982 ml/min/m² (Table 1). The individual Cl_T values ranged from 562 to 1786 ml/min/m².
5. The terminal phase generally started at 18 to 30 hr after dosing and corresponded to 30.36% of the area under the plasma concentration-time curve (Table 2).
6. The mean central compartment volume of distribution, V_1 , was approximately 275% of body weight indicating that the drug is highly bound to rapidly perfused tissues (Table 3).
7. The volume of distribution at steady state was extremely large ($Vd_{ss} = 434$ L/m²). The total body volume of distribution ($Vd_T = 1113$ L/m²) was substantially greater than Vd_{ss} indicating a very high degree of tissue binding of the drug (Table 3).
8. The smallest of the microscopic rate constants was k_{31} (data not presented) suggesting that release of the drug from deep tissue binding sites is the rate determining process for elimination of the parent drug from the body.
9. Within the dose range 92.0 to 800.0 mg/m², there was no apparent dependence of total body clearance or volume parameters on dose (Tables 1 and 3). The pharmacokinetic rate constants and Vd_T for the one patient at 800.0 mg/m², studied thus far, were markedly different from the values determined for patients treated with lower doses, however, the total body clearance in this patient was similar to the mean value for all patients.

10. Amonafide plasma concentration-time profiles determined on days 5-7 for 12 patients on the multiple dose schedule (38.0-121.6 mg/m²/day x 5) followed similar three compartment open model behavior: $t_{1/2\alpha} = 11.3$ min; $t_{1/2\beta} = 2.40$ hr; $t_{1/2\gamma} = 13.04$ hr and MRT = 7.04 hr (data previously presented).
11. The mean total body clearance in the multiple dose study was 1085 ± 255 ml/min/m² (data previously presented).
12. None of the multiple dose mean pharmacokinetic parameters was significantly different from the corresponding mean parameters determined during the single dose schedule.
13. The mean urinary excretion of the parent drug for 26 patients on the single schedule was 11.9% of the dose and the mean renal clearance was 111.7 ml/min/m² (data previously presented). A wide range of renal clearance values was observed (7.7-294.9 ml/min/m²). A significant number of the patients exhibited renal clearance which was greater than the glomerular filtration rate suggesting that there is active secretion of the parent drug. No evidence was obtained for the conversion of a metabolite to the parent drug in urine.
14. Amonafide is extensively metabolized in man. Amonafide and its basic metabolites were isolated from patient urine using an Amberlite XAD-2 resin column followed by preparative thin layer chromatography. Six metabolites were obtained (Table 4). The structures of these compounds were derived from PMR and mass spectra. The structure of one metabolite, 5-(N-acetyl)-6-methoxyamonafide, was incorrectly tabulated in the July, 1986 report. Synthetic samples of 5 of the compounds were prepared.
15. Four peaks corresponding to metabolites were observed in chromatograms of alkalized plasma extracts. Comparative and co-chromatography indicate that these metabolites are 5-(N-acetyl)-amonafide, noramonafide, 5-(N-acetyl,N-hydroxy)-amonafide, and 5-(N-acetyl)-amonafide N-oxide. The principal metabolites, based upon the fluorescence intensity of the chromatographic peaks, are thought to be 5-(N-acetyl)-amonafide and noramonafide.
16. Three urinary metabolites which have not been observed in plasma are amonafide N-oxide, 5-(N-acetyl)-6-methoxyamonafide and 5-amino-1,3-dioxo-1H-benz(de)isoquinoline-2(3H)-acetic acid. The latter compound is the 5-amino analog of the aldose reductase inhibitor alrestatin and was isolated from patient urine as the methyl ester.
17. In general, amonafide is highly bound to tissue binding sites and the rate of release of the drug from these sites is slow. The drug is extensively metabolized and two major metabolic routes are N-acetylation and N-demethylation. A study of polymorphic acetylation in these patients is complicated by the further metabolism of 5-(N-acetyl)-amonafide. The pharmacokinetics of amonafide are not dose-dependent and no significant accumulation occurs during multiple dosing.

TABLE 1

AMONAFIDE MEAN TOTAL BODY CLEARANCE AND HARMONIC
MEAN HALF-LIVES AND RESIDENCE TIMES AT EACH DOSE
LEVEL FOLLOWING SINGLE IV ADMINISTRATION

Patients	Dose	$t_{1/2\alpha}$	$t_{1/2\beta}$	$t_{1/2\gamma}$	MRT	Cl_T
n	(mg/m ²)	(min)	(hr)	(hr)	(hr)	(ml/min/m ²)
5	92.0	15.7	2.58	10.60	6.13	793
4	128.8	8.1	2.02	10.93	7.37	994
4	165.6	15.2	2.47	10.84	7.18	895
3	220.8	6.2	2.50	10.12	7.01	1055
5	294.4	20.7	3.12	13.37	7.20	1102
3	392.5	18.6	1.56	8.42	6.20	979
4	523.4	31.9	2.93	18.14	7.47	968
3	697.8	31.9	3.05	12.10	6.24	1152
1	800.0	88.3	6.58	23.14	8.02	972
Mean (n=32)	-	20.7	2.69	12.33	6.91	982

TABLE 2

MEAN PERCENTAGE OF THE AREA UNDER THE AMONAFIDE
PLASMA CONCENTRATION - TIME CURVE CORRESPONDING
TO EACH OF THE TRI-EXPONENTIAL DECAY PHASES AT
EACH DOSE LEVEL FOLLOWING SINGLE IV ADMINISTRATION

Patients	Dose	Alpha Phase	Beta Phase	Gamma Phase
n	(mg/m ²)	%	%	%
5	92.0	20.76	53.40	25.84
4	128.8	15.79	44.38	39.82
4	165.6	20.63	43.73	35.64
3	220.8	14.95	45.59	39.46
5	294.4	21.35	53.97	24.69
3	392.5	13.47	44.95	41.58
4	523.4	27.84	50.64	21.53
3	697.8	39.21	33.82	26.96
1	800.0	42.47	50.59	6.94
Mean (N=32)	-	22.28	47.36	30.36

TABLE 3

MEAN AMONAFIDE VOLUME PHARMACOKINETIC PARAMETERS
AT EACH DOSE LEVEL FOLLOWING SINGLE IV ADMINISTRATION

Patients (n)	Dose (mg/m ²)	V ₁ (L/m ²)	V ₂ (L/m ²)	V ₃ (L/m ²)	Vd _{ss} (L/m ²)	Vd _γ (L/m ²)
5	92.0	71.5	108.2	127.3	307.0	872.9
4	128.8	64.3	149.7	260.0	474.0	963.7
4	165.6	91.9	122.7	184.9	399.5	845.1
3	220.8	80.3	223.2	202.7	506.2	945.3
5	294.4	123.7	168.1	207.9	499.5	1311.4
3	392.5	83.2	113.8	193.0	389.9	860.8
4	523.4	134.1	141.4	195.3	470.8	1596.6
3	697.8	132.1	97.8	206.2	436.1	1238.8
1	800.0	228.4	154.3	85.3	468.0	1947.9
Mean (n=32)		101.6	140.5	191.5	433.6	1113.3

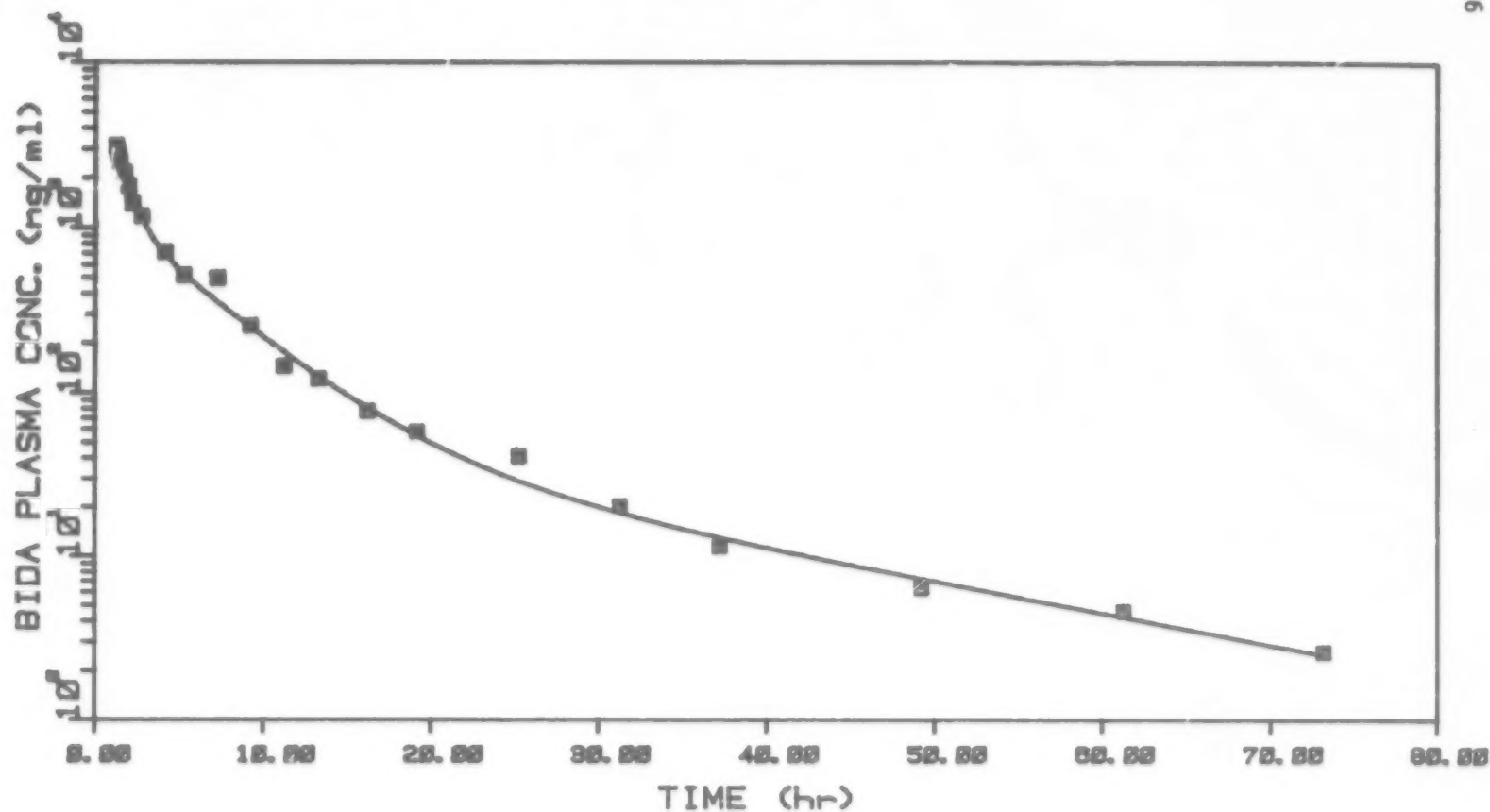
TABLE 4

AMONAFIDE METABOLITES ISOLATED FROM URINE USING
XAD-2 RESIN FOLLOWED BY PREPARATIVE TLC

METABOLITE	TLC (R _f)	SYNTHETIC COMPOUND PREPARED	500 MHz PMR	M.S.
noramonafide	0.14	x	x	CI,EI
5-(N-acetyl)-amonafide N-oxide	0.20	x	x	CI
amonafide N-oxide	0.24	x	x	CI
5-(N-acetyl,N-hydroxy)- amonafide	0.27	x	a	CI
amonafide	0.42		x	CI,EI
5-(N-acetyl)-amonafide	0.42	x	b	CI,EI
5-(N-acetyl)-6-methoxy- amonafide	0.71		x	c

^aPMR of metabolite. ^bPMR of metabolite with amonafide.
^cDecomposed in source.

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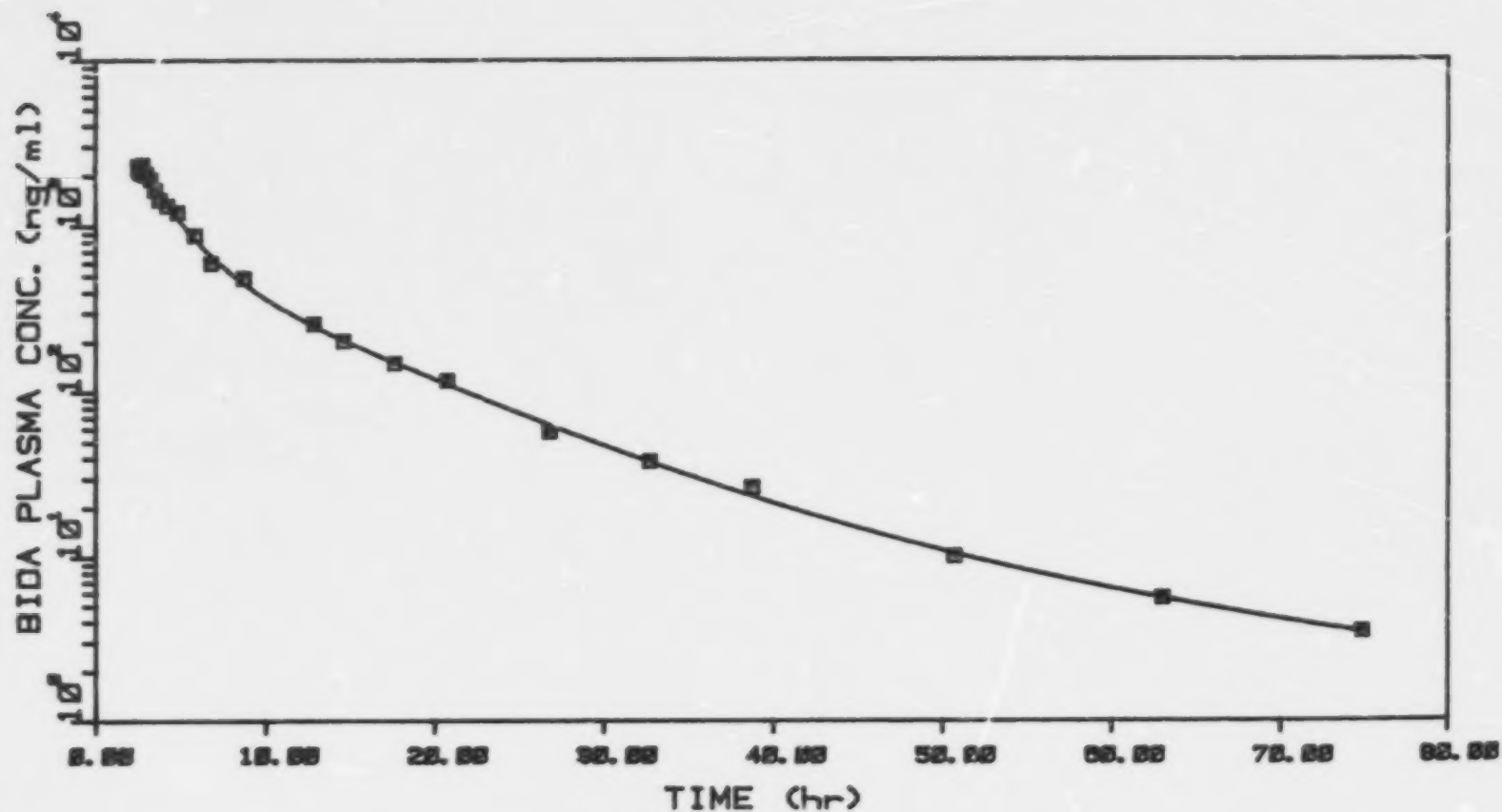


PATIENT R. L.

DOSE = 697.8 MG/METER SQ. BIDA

HALF-LIFE = 15.62 HOURS

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PATIENT R.R.
DOSE = 800 MG/METER SQ. BIDA
HALF-LIFE = 23.14 HOURS

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BACKGROUND

- Benzisoquinolinedione is a novel synthetic derivative of 3-nitro-1,8 naphthalic acid.
- It inhibits DNA synthesis and functions as a DNA intercalator.
- It is active against leukemias L1210 and P388, B-16 melanoma and murine sarcoma M5076. Best ILS (110-144%) occurred with multiple dosing, (Days 1-5; 1-9; 1,5,9).
- The LD10 in mouse was $180 \text{ mg/m}^2 \times 1$ or $75 \text{ mg/m}^2/\text{d} \times 5$; 1-2 x MELD10 in dog was nontoxic.

METHODOLOGY

Patients - Adults with metastatic solid tumors, PS 0-3, failed standard therapy, normal hepatic and renal function and normal blood counts.

Drug Schedule - Starting dose, $1/10 \text{ LD10} = 10 \text{ mg/m}^2/\text{d} \times 5 \text{ days q } 3 \text{ wks}$. Dose escalated 50%-100% to grade 2 toxicity. Drug mixed in normal saline, infused over 30-60 minutes.

Monitoring - Blood counts twice/weekly, SMA, urinalysis q 1-2 weekly, tumor measurements q 3-4 weeks.

Pharmacokinetics in selected patients.

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PHASE I STUDY OF BENZISOQUINOLINEDICINE (BIDA)

PATIENT CHARACTERISTICS

Number of Patients Entered:	28
Age, median (range):	51 (25-67)
Sex:	
Female	11 (39%)
Male	17 (61%)
Performance Status:	
0,1	19 (68%)
2,3	9 (32%)
Prior Therapy:	
Chemotherapy	13 (46%)
Radiation and Chemotherapy	15 (54%)
Prior Chemotherapy:	
Number of Regimens:	
1	6 (21%)
2	9 (32%)
3	13 (47%)

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PHASE I STUDY OF BIDA

Tumor Types

Colorectal Carcinoma	8
Lung	8
Sarcoma	4
Melanoma	3
Breast	2
Head and Neck	1
Esophagus	1
Unknown 1 ⁰	1

BIDA: DOSE ESCALATION SCHEME

Dose mg/m ²	No. of Patients	No. of Courses		
		Initial	Subsequent	Total
10	3	3	0	3
20	7	4	3	7
30	3	1	2	3
45	5	2	3	5
70	4	0	4	4
110	4	2	2	4
170	4	1	3	4
260	6	3	3	6
400	13	10	11	21
500	6	4	3	7
625	1	0	2	2

PHASE I STUDY OF BIDA

HEMATOLOGIC EFFECTS

Dose Level mg/mm ² x 5	No. Evaluable Courses	Lowest Median Counts X 10 ³ /mm ³	
		Absolute Granulocyte Count (Day) (Range)	Platelet (Day) (Range)
10-170	30	4.0 (12) (2.2-10.6)	309 (11) (197-572)
260	6	3.8 (15) (0.8-15.7)	265 (13) (71-560)
400	21	0.8 (15) (0-4.4)	130 (15) (5-200)
500	7	0.3 (15) (0-2.3)	103 (15) (6-239)
625	2	1.5 (15) (1.3-1.7)	197 (13) (191-203)

NON-HEMATOLOGIC TOXICITY OF BIDA

	Dose mg/mm ² /day x 5 days						Total
	10-170	260	320	400	500	625	
Evaluable Courses	30	6	4	21	7	2	70
Nausea/Vomiting	1	1	1	16	5	2	26
Dizziness	-	1	-	10	4	1	16
Diaphoresis	-	1	-	7	2	-	10
Skin Rash	-	-	-	4	1	-	5
Tinnitus	-	-	1	3	1	-	5
Phlebitis	1	-	-	2	-	-	3

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CLINICAL TOXICITY OF BIDA

Number of Evaluable Patients	27
Nausea/Vomiting	19 (70%)
Dizziness/Vertigo	12 (44%)
Diaphoresis	9 (33%)
Ear Fullness/Tinnitus	7
Skin Rash	5
Alopecia	4
Phlebitis	2
Dyspnea	1

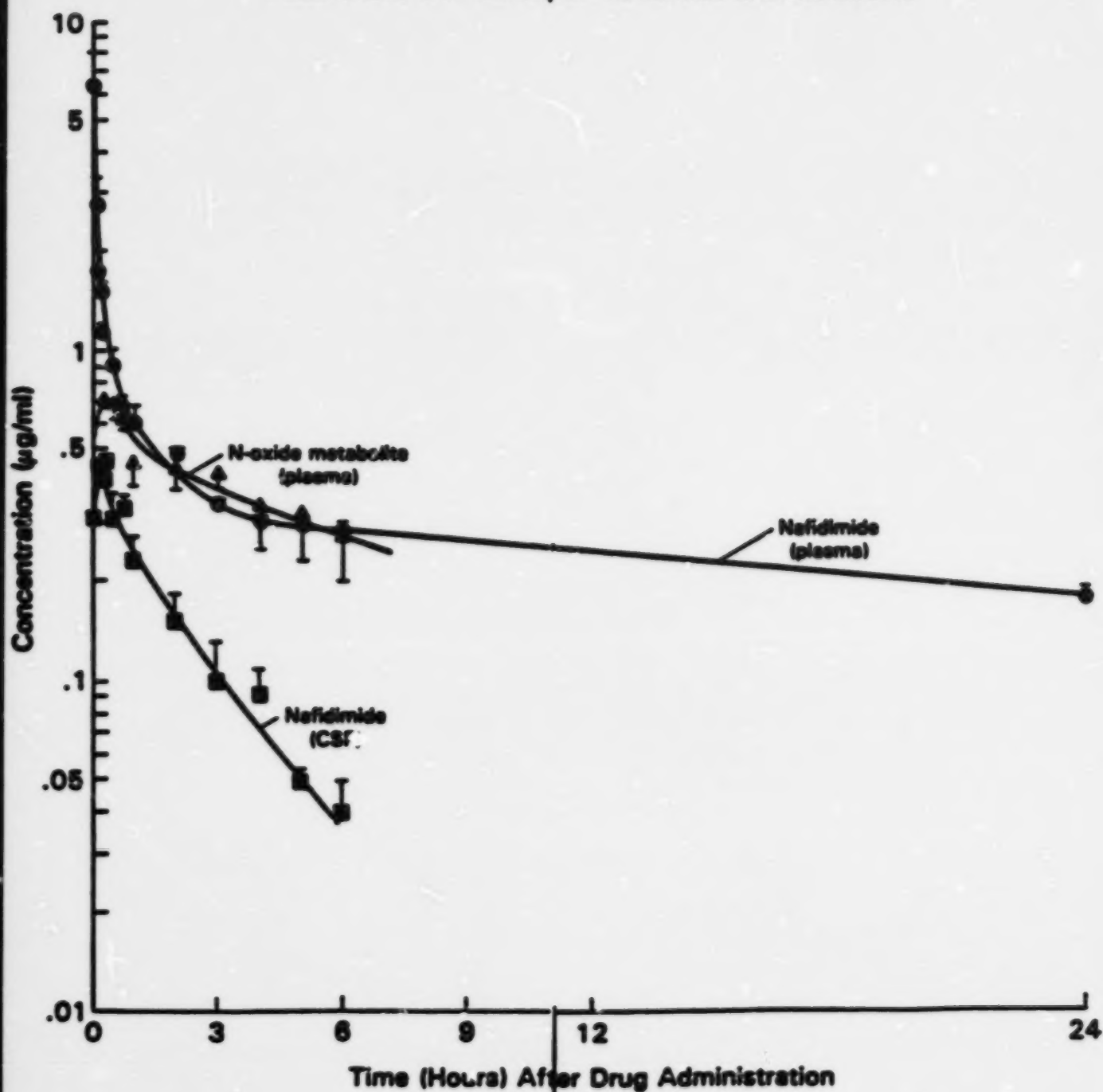
ANTITUMOR EFFECTS OF BIDA

Number of Evaluable Patients	24
Complete Response	0
Partial Response	0
Minor Response	1 (Lung CA)
Stable Disease	4 (Lung-3, Esoph-1)
Progression	19

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FIGURE 3 Plasma Pharmacokinetics of Nafidimide In The Dog

Plasma and CSF Levels of Nafidimide and Metabolite



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FIGURE 1

Analytical Methodology

HPLC: J. Chromatography (Biomed. Applications)

345: 408-412, 1985

Thermospray LC/MS: Courtesy of Vestech, Inc.
Houston, Texas

FIGURE 2

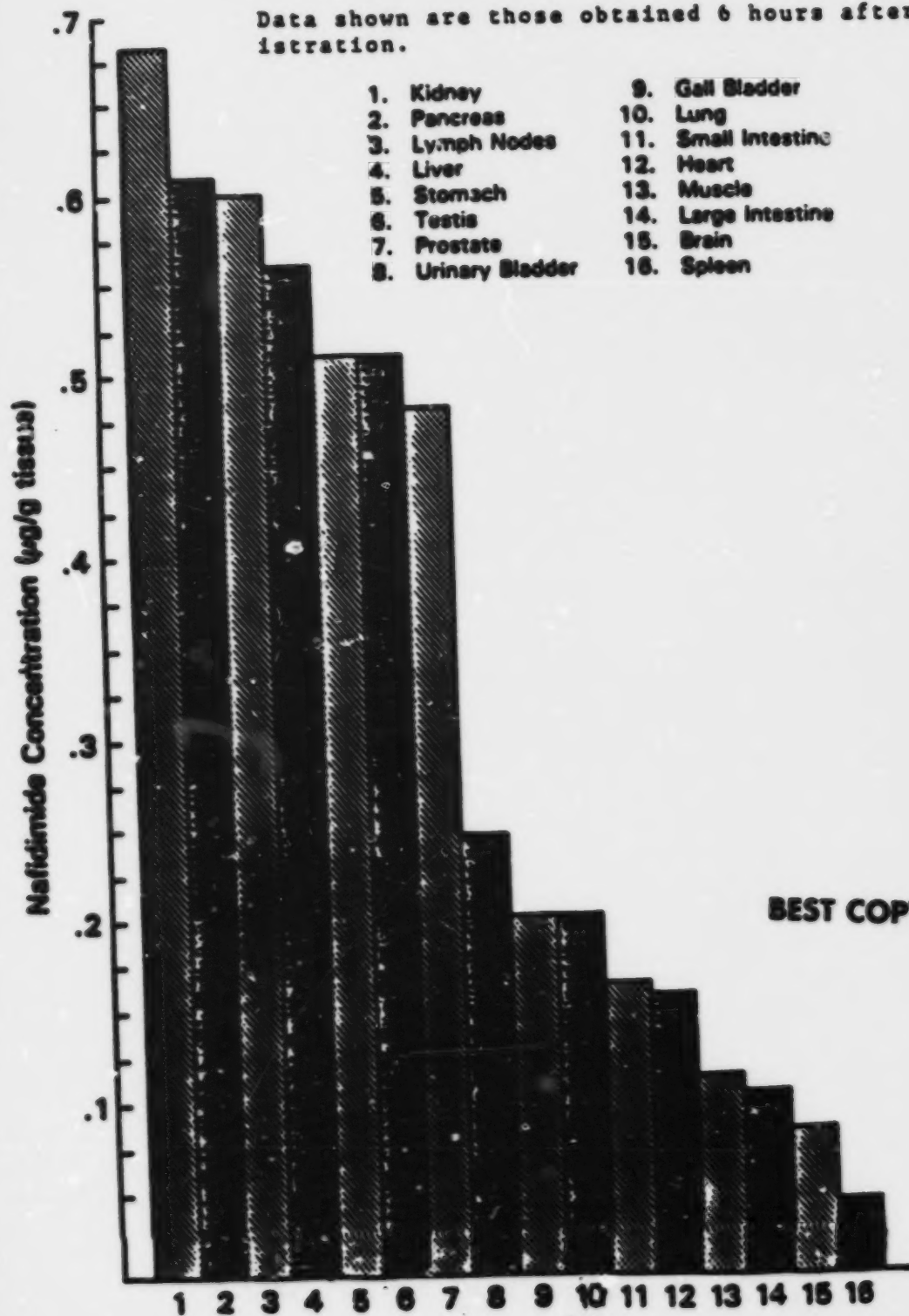
Preclinical Pharmacology: Mechanism(s) of Drug Action

1. Amonafide is an intercalating agent.
2. Produces both single and double strand breaks
3. Cellular drug uptake is rapid and reaches a steady state at 30 min at 37° C. Drug egress is rapid.
4. Since drug egress occurs at a rate similar to repair of DNA lesions, prolonged drug exposure may be required for optimal antitumor activity.

Figure 6

Tissue Concentrations of Nafidimide (6h) in the dog.

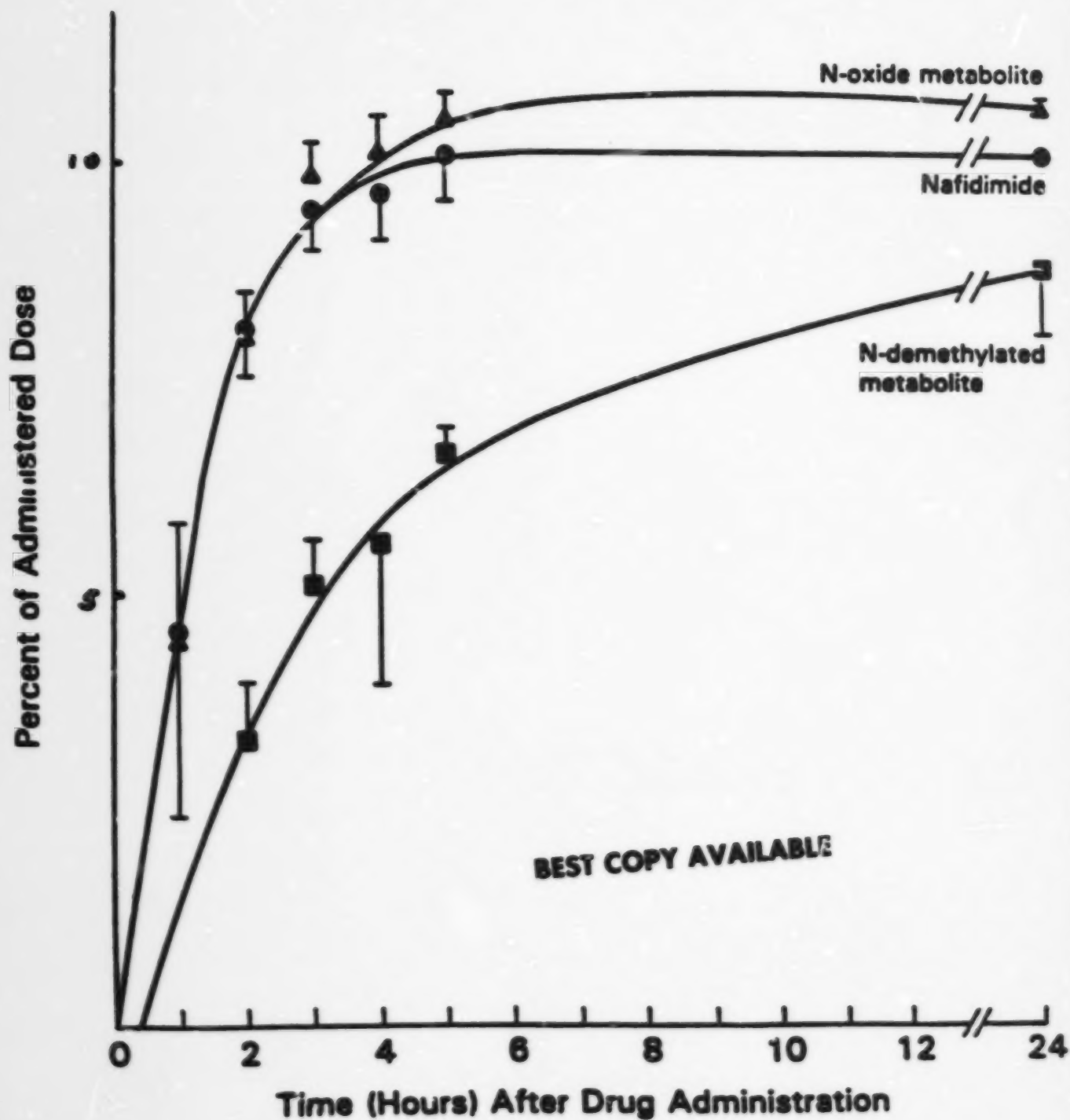
Data shown are those obtained 6 hours after drug administration.



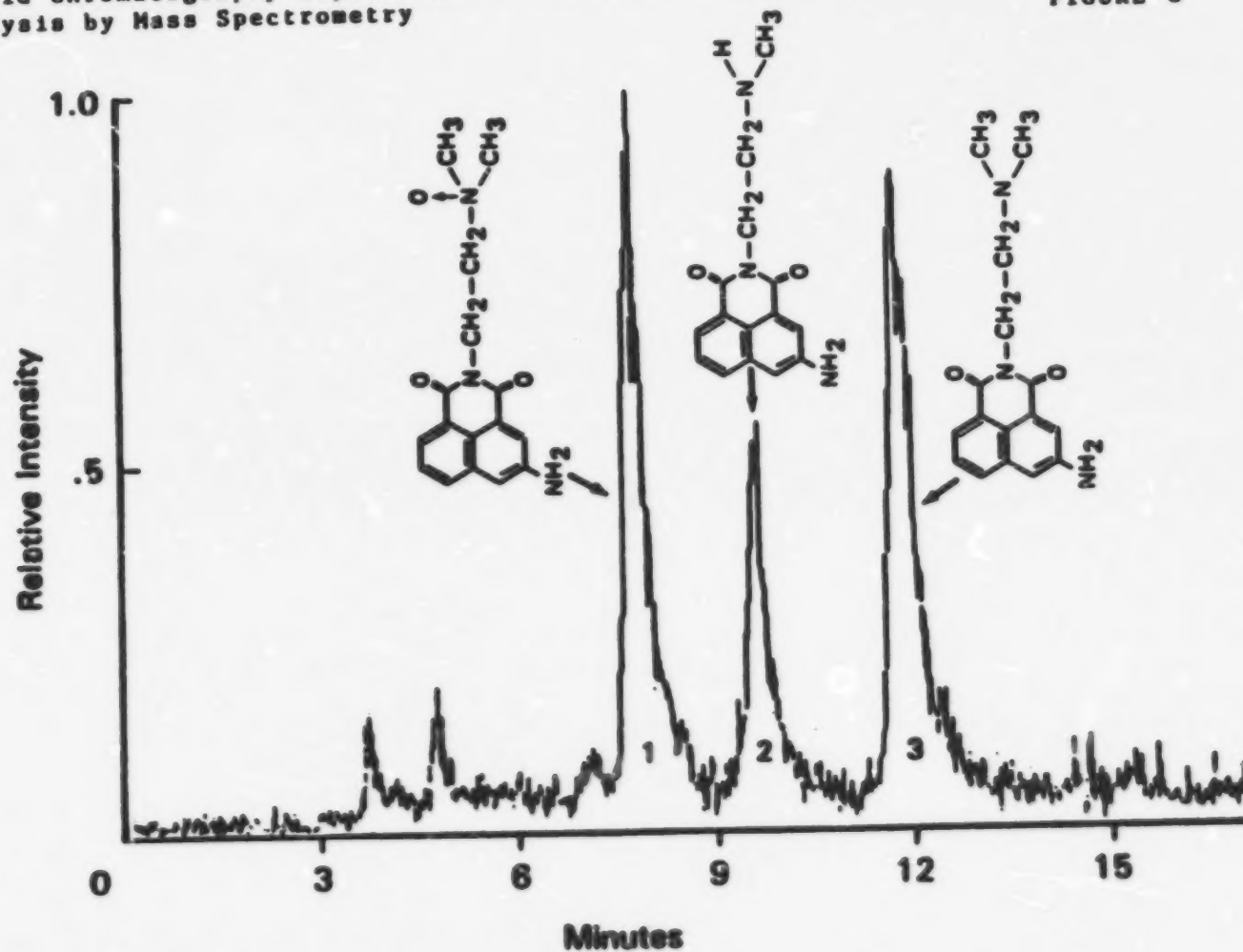
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FIGURE 4

Cumulative Urinary Excretion of Nafidimide and Metabolites



Liquid Chromatography Separation of Nafidimide and Metabolites Prior To
Analysis by Mass Spectrometry FIGURE 8



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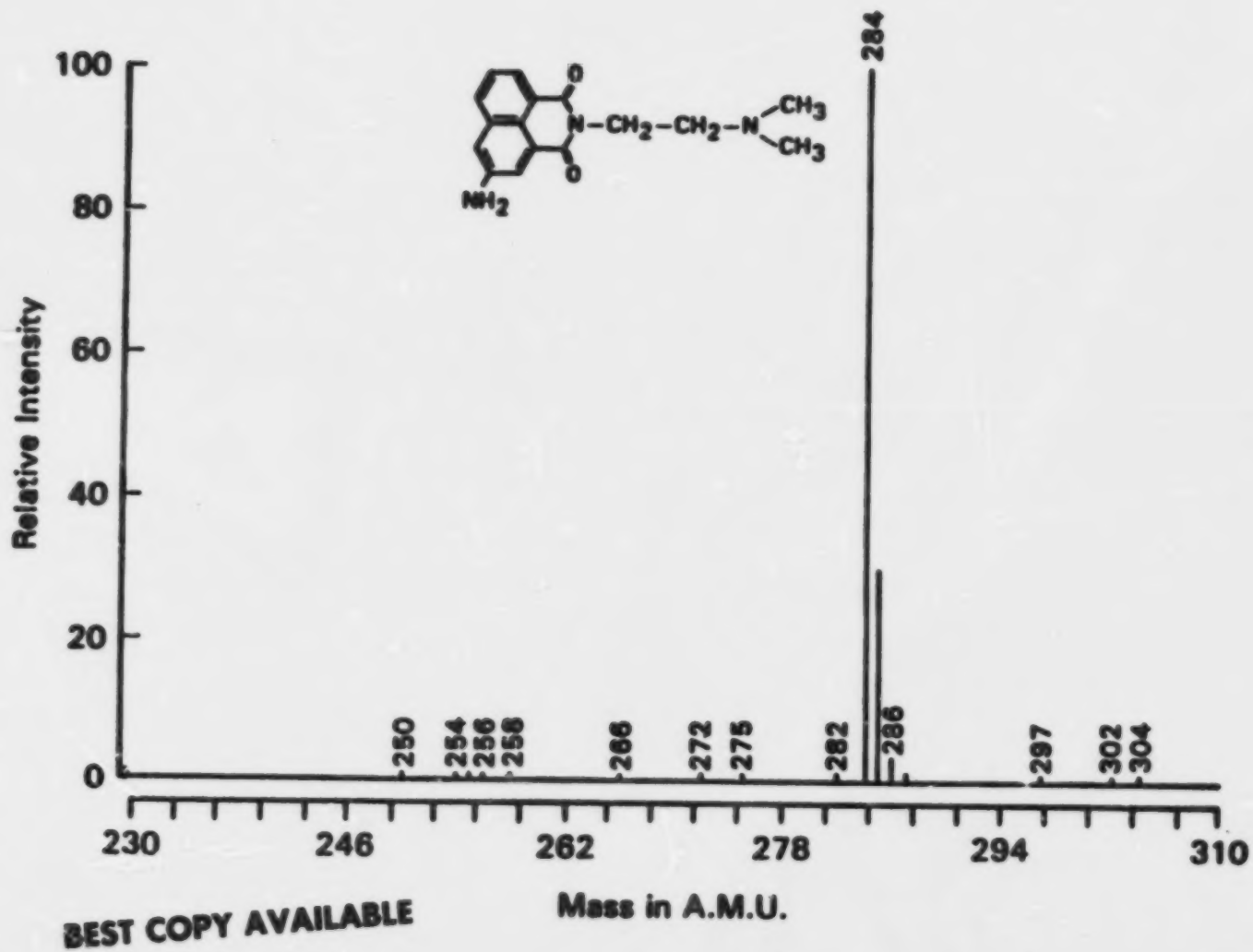
Amonafide
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M.D. Anderson
November 1986

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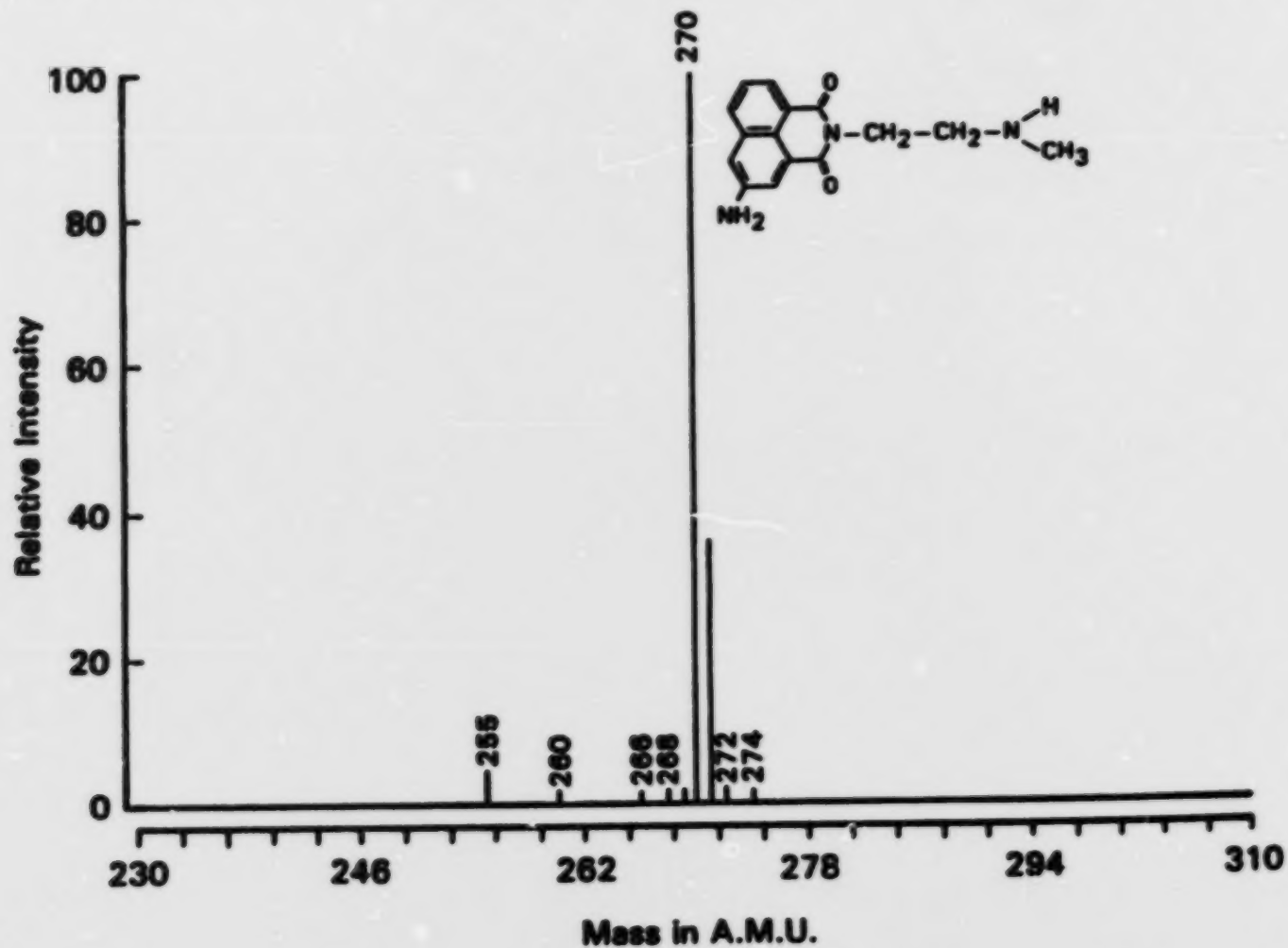
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FIGURE 9

Mass Spectrometry Data Obtained From Nalidiximide Isolated From Dog Urine



Mass Spectrometry Data Of N-Demethylated Nafidimide Isolated From Dog Urine FIGURE 10



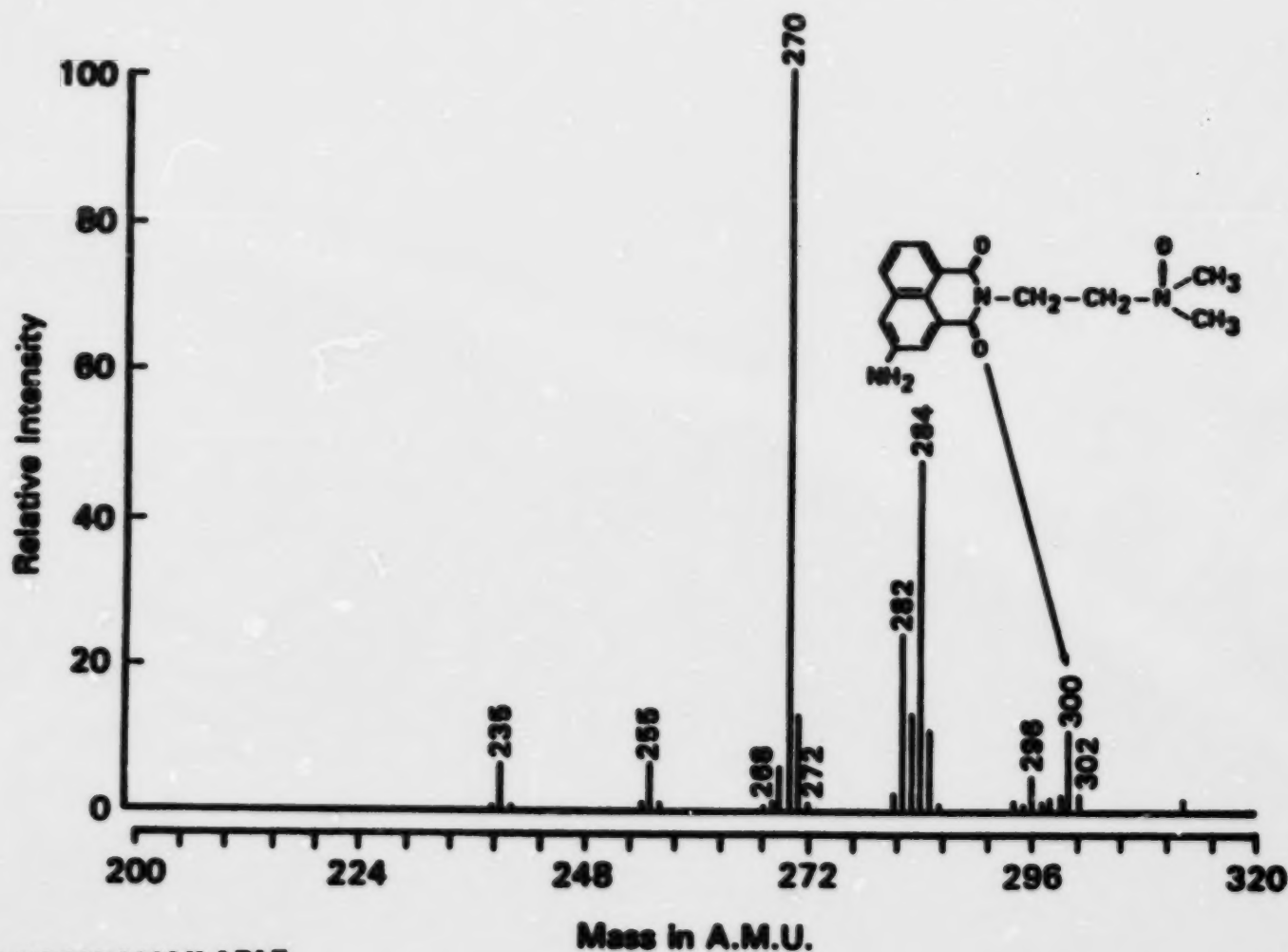
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November 1986

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FIGURE 11

Mass Spectrometry Data Of N-Oxide Nafidimide Isolated From Dog Urine



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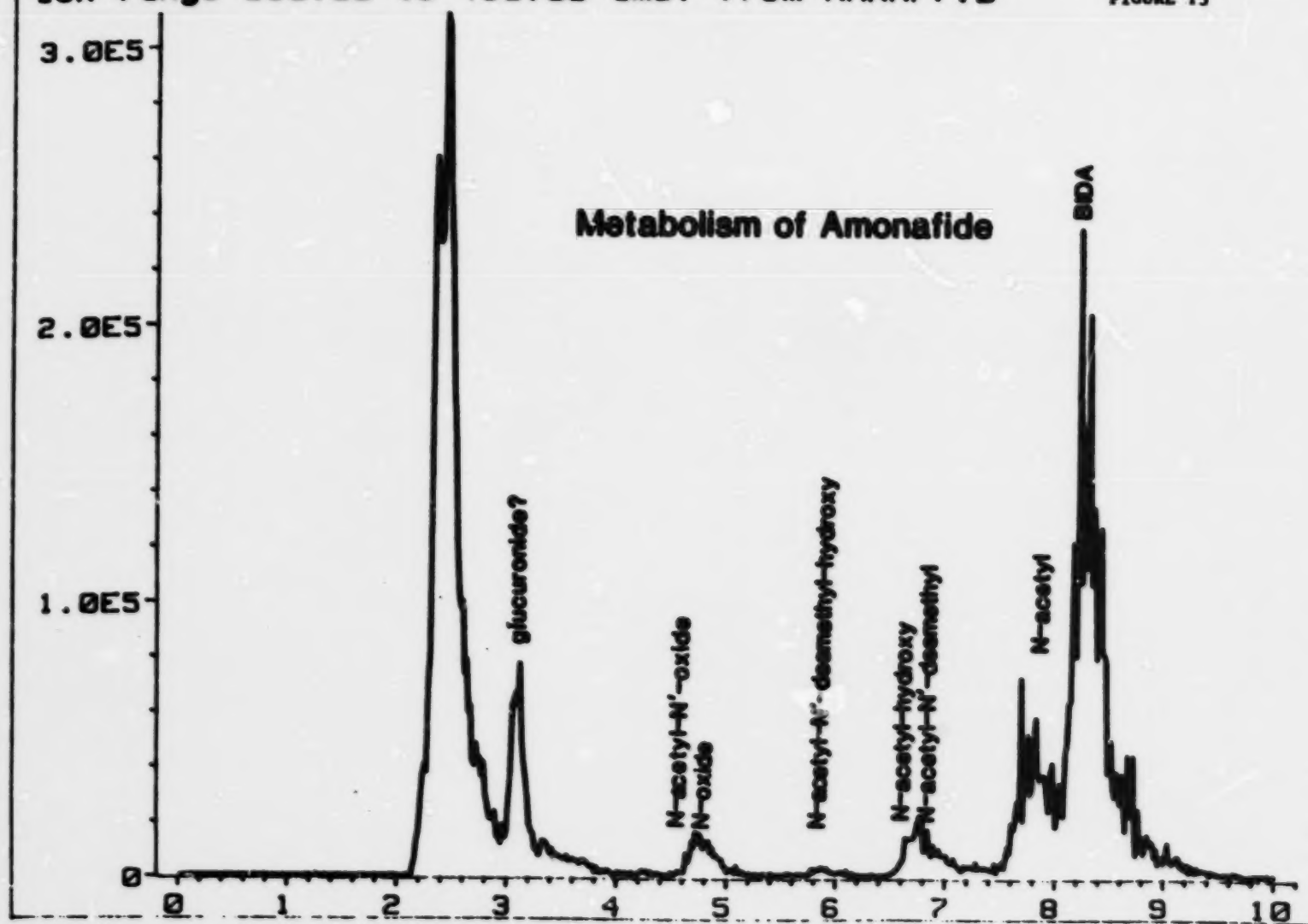
FIGURE 12

Species-Dependent Metabolism of Amonafide

1. Major metabolites in the dog:
N-desmethyl
N-oxide
N-acetylated species not observed
2. Major metabolites in humans:
N-acetylated derivatives
Few N-desmethylated species observed

Ion range 260.00 to 450.00 amu. from MMNAF7.D

FIGURE 13



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FIGURE 15

Clinical Pharmacology of Amonafide

1. Amonafide metabolism in the dog differs from that in humans.
2. Metabolism of amonafide in humans shows considerable patient-to-patient variation.

Clinical Aronafide Pharmacokinetic Parameters^a

Patient	$V_d (L/m^2)$	$t_{1/2} (hr)$	$AUC \left(\frac{ng \cdot hr}{L} \right)$	$CL_T \left(\frac{L}{m^2 \cdot hr} \right)$
1	42.1	1.9	26.6	15.1
2	141.2	3.8	15.5	25.6
3	199.4	4.5	16.4	24.3
4	109.9	2.4	13.0	30.7
5	107.0	2.0	10.9	36.6
6	124.2	2.6	11.9	33.1
7	374.8	7.1	11.0	36.6
Mean $\pm$ SD	151.2 $\pm$ 105.2	3.5 $\pm$ 1.9	15.0 $\pm$ 5.5	28.9 $\pm$ 7.8

^aDose = 400 mg/m²

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Today I will be reporting our results to date with our phase I study of Amonafide given by 24-hour continuous intravenous infusion every four weeks. Our interest in the 24-hour infusion schedule was based on pre-clinical data suggesting schedule dependence versus L-1210 leukemia in which a single intravenous dose of the drug produced a 37% increase in life span; however, the same dose given intraperitoneally in 8 administrations over 24 hours produced a 74% increase in the life span of these animals. Our choice of a starting dose was based on the fact that a dose of 18.4 mg/m² or one-tenth of the mouse equivalent LD10 produced some transient renal and hepatic toxicity. We, therefore, chose a starting dose of 1/30th of the mouse equivalent LD10 or 6.1 mg/m² of body surface area. The requirements for entry into this study are shown in the next slide and include the usual requirements for adequate hematologic, hepatic, and renal function. Other criteria included the absence of any central nervous involvement by malignancy and good performance status. To this date 15 patients have been entered on study, 10 men and 5 women. Their histologic types are shown on this slide and include a large proportion of adenocarcinoma of the colon, typical of the patients in our phase I studies.

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Amonafide

T85-0221

Mayo Clinic

November 1986 There were a number of relatively uncommon tumors included in this group as

well.

The next slide shows the dose escalation scheme, the number of new patients entered at each level, and the number of courses of treatment given at each level. I should point out that after we had entered three patients at the 18.4 mg/m² level, we were informed that groups investigating other schedules were administering the drug at much higher doses and we felt that we could modify our escalation scheme. Accordingly, no new patients were entered at the 36.8 or 73.6 mg/m² level and our next level of escalation was at 100 mg/m². We have entered three new patients at the 200 and 400 mg/m² levels without significant hematologic toxicity. However, the patient treated at the 400 mg/m² level who developed a nadir white blood cell count of 2,900 subsequently had her next course escalated to 600 mg/m² and developed significant myelosuppression and also had gastrointestinal bleeding with melanic stools. Her white blood cell count and platelet count recovered over a period of just a few days and she was treated with red cell transfusions. She declined further gastrointestinal evaluation for a bleeding site. When she returned for consideration of a

subsequent course of treatment, she had definite progression on a liver scan and was not given a fourth course of therapy. To date we have not seen any objective responses and we have had no indication of any hepatic, renal, or gastrointestinal toxicity. We have opened the study again for accrual of 3 new patients at a dose of 600 mg/m² of body surface area.

Phase I Study of Nafidimide by 24 hr Infusion

Pharmacokinetic Study

Assay

Nafidimide and its metabolites are measured by reversed phase high performance liquid chromatograph (hplc) assay where plasma is injected indirectly onto the hplc without the need for prior extraction (Powis et al., J. Chromatogr. 345:402-407, 1985). The lower limit for detection of nafidimide in plasma is 2.5 ng/ml.

Patient Pharmacokinetics

Pharmacokinetics of nafidimide have been studied in 10 patients at doses of 6.1 to 400 mg/m² given by 24 hr infusion. Plasma plateau concentrations were attained by 24 hr. Figure 1 shows plasma concentrations of nafidimide in a patient during infusion and Figure 2 in the same patient after infusion of nafidimide. The half-life for elimination of nafidimide calculated from the infusion phase in this patient was 7.0 hr and from the elimination phase 7.8 hr.

Table 1 shows the steady state levels and total body clearance for nafidimide in the 10 patients so far studied. These clearances are in the range reported for patients receiving the drug by bolus injection. There appears to be a considerable range of clearance values (170-926 ml/min/m²) between patients.

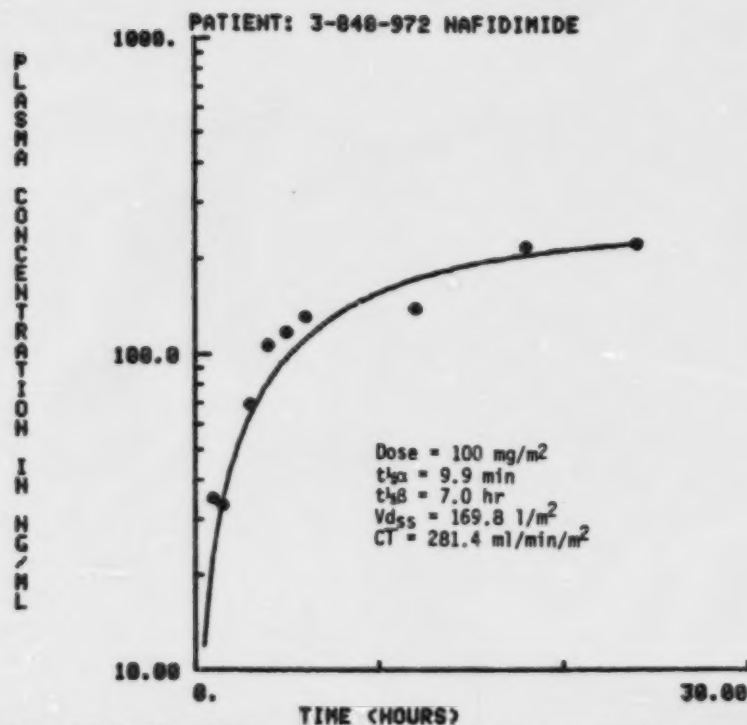


Figure 1. Plasma concentrations of nafdinide during 24 hr infusion at a dose of 100 mg/m². Points are the observed values, the continuous line is the computer fit to the data.

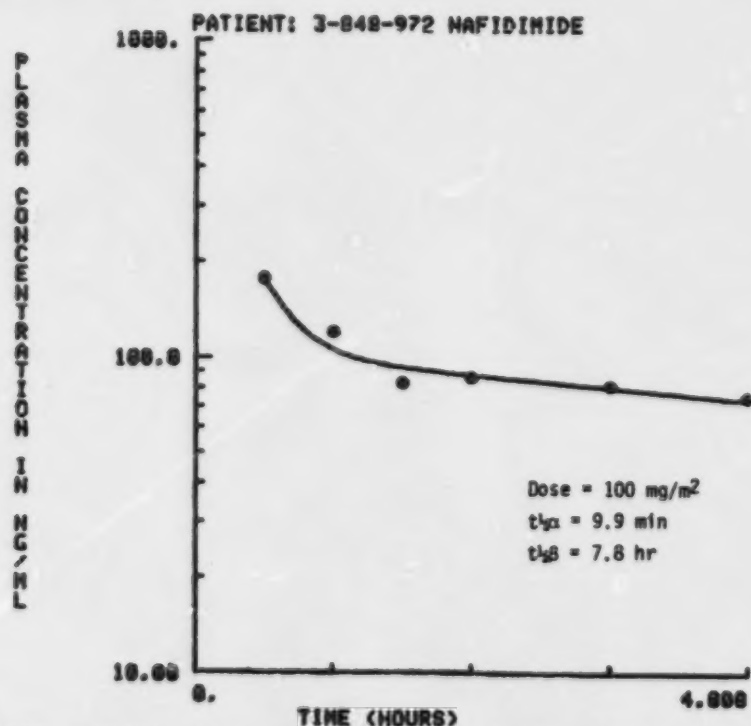


Figure 2. Plasma concentrations of nafdinide following a 24 hr infusion at a dose of 100 mg/m² in the same patient as Figure 1. Points are the observed values, the continuous line is the computer fit to the data.

Pharmacokinetics of Nafidimide
Given by 24 hr Infusion

Patient	Dose mg/m ²	C _{ss} ng/ml	Cl ml/min/m ²	24 hr urine %
1	6.1	25	170	2.8
2	18.4	25	506	0.2
3	100	247	347	3.6
4	100	200	281	1.8
5	200	150	926	2.0
6	200	180	772	2.9
7	200	450	309	-
8	400	550	505	4.0
9	400	1500	185	7.7
10	400	300	926	1.1

Urinary excretion of nafidimide was 0.2 to 7.7% of the dose over 24 hr (during the infusion). The relative amount excreted may be increased at higher doses suggesting there could be some saturation of a reabsorption process. The total amount of nafidimide excreted in urine is, however, small.

Metabolites

Plasma shows at least two polar metabolites (Figure 3). Urine shows at least eight and possibly more fluorescent peaks (4 major and 4 minor) that may be metabolites (Figure 4). The metabolites have not been identified.

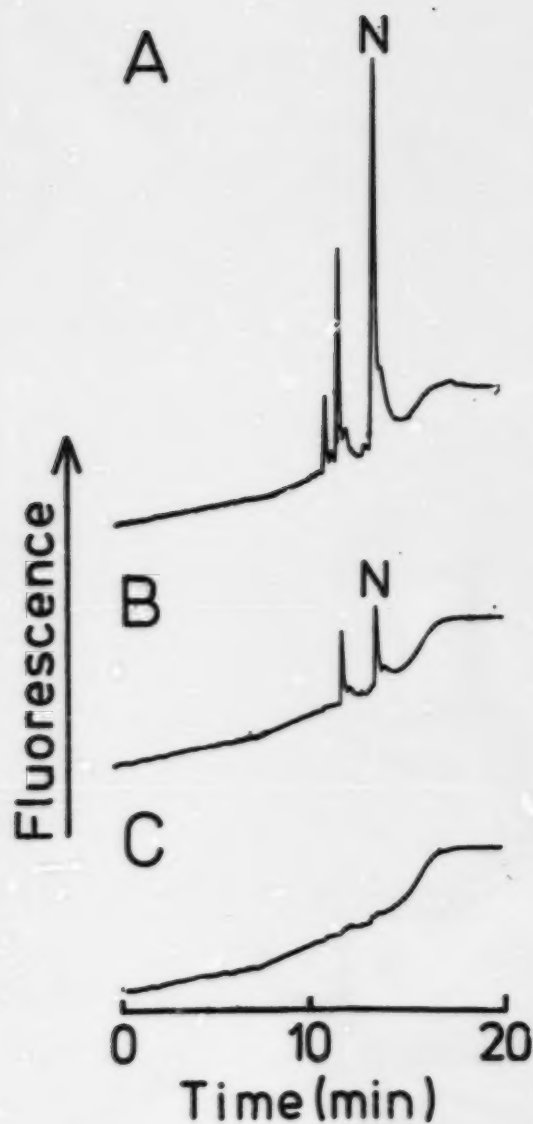


Figure 3. Chromatograms of plasma from patient receiving a 24 hr infusion of nafidimide 400 mg/m². C, zero time; B, 1 hr; A, 24 hr. N is nafidimide.

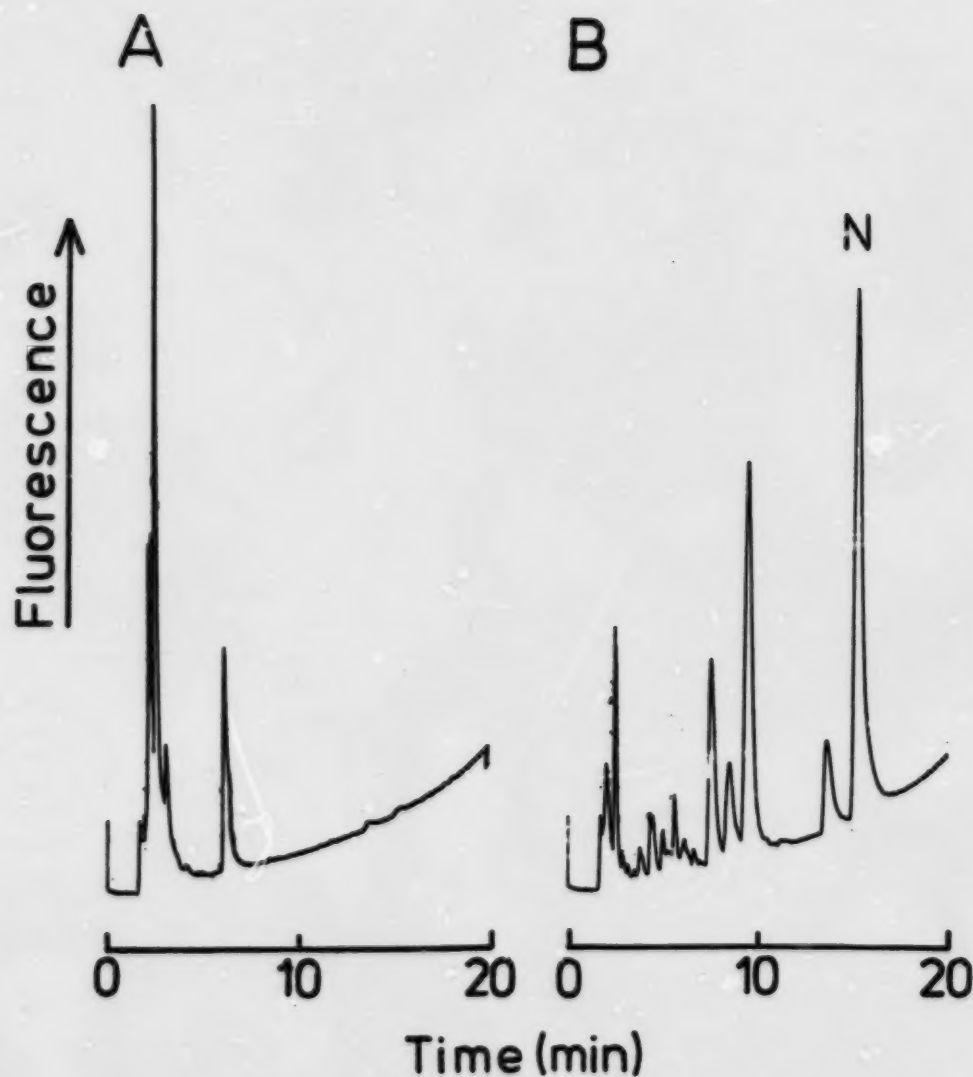


Figure 4. Chromatograms of urine from a patient receiving a 24 hr infusion of nafidimide 400 mg/m². A, control urine sample; B, urine collected over 24 hr of drug infusion.

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Mayo Clinic
Phase I Study of Amonafide
24 hour continuous intravenous infusion
every 4 weeks

Choice of 24⁰ Infusion Schedule

- schedule dependence vs L12 10
- single IV dose produced 37% increase lifespan
- same dose IP in 8 doses/24 hours produced 74% increase lifespan

Choice of Starting Dose

- single day MELD-10 = 18.4 mg/m²
- transient renal and hepatic toxicity seen in animals at this dose
- starting dose 1/30 MELD-10 = 6.1 mg/m²

1. Advanced cancer beyond cure or effective palliation with standard therapy.
2. Adequate hematologic status
WBC $\geq$ 4000, HGB $\geq$ 10, PLTS $\geq$ 130,000
3. Adequate hepatic function
non-elevation of direct bilirubin,
alkaline phosphatase, or SGOT $<$ 3x normal
4. Adequate renal function
creatinine $\leq$ 1.5
5. Performance status 0, 1, 2
6. No CNS metastases
7. No significant heart disease
8. Recovery from prior therapy
9. Informed consent

15 patients entered to date

M/F 10/5

Histologic Types

Colon ACA 7
Renal ACA 1
Breast ACA 1
Adrenocortical ACA 1
Melanoma 1
Cylindroma 1
Leiomyosarcoma 2
Hemangiopericytoma 1

Dose mg/m²

New pts entered/# of courses

6.1	3/3	
18.4	3/4	
36.8	0/2	
73.6	0/2	
100.0	3/4	
200.0	3/6	nadir WBC 3.0 1 pt
400.0	3/8	nadir WBC 2.9 1 pt
600.0	0/2	nadir WBC 1.5, plts 42
		GI bleeding - 1 pt
720.0	0/2	

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FLAVONE ACETIC ACID
PATIENT CHARACTERISTICS

NUMBER PATIENTS	31
MEDIAN AGE	46 (RANGE 20-79)
INITIAL PERFORMANCE STATUS (PS)	
PS	NUMBER OF PATIENTS
0	11
1	10
2	2
3	8
4	0
PRIOR THERAPY	
CHEMOTHERAPY	25
RADIATION THERAPY	11

FLAVONE ACETIC ACID
DISEASE CATEGORY

COLORRECTAL ADENOCARCINOMA	19
HEAD AND NECK CARCINOMA	3
LUNG CARCINOMA	2
KIDNEY CARCINOMA	1
OVARIAN CARCINOMA	1
MALIGNANT MELANOMA	1
PANCREATIC CARCINOMA	1
NEUROBLASTOMA	1
SARCOMA	1
UNKNOWN PRIMARY	1

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FLAVONE ACETIC ACID

DRUG ADMINISTERED

DOSE LEVEL (mg/m2)	NUMBER OF PATIENTS	NUMBER COURSES
100	3	5
400	5	18
1000	8	21
1500	5	10
2000	5	7
2700	4	9
3600	5	9
4800	3	4

FLAVONE ACETIC ACID

HEMATOLOGIC TOXICITY

DOSE LEVEL (MG/M2)	NUMBER PATIENTS	NUMBER COURSES	MEDIAN WBC* NADIR	WBC RANGE
100	3	5	5.8	4.9 - 8.4
400	5	18	5.3	4.5 - 12.5
1000	8	21	5.4	3.1 - 11.6
1500	5	10	6.6	3.6 - 13.2
2000	5	7	6.4	1.9 - 10.1
2700	4	9	3.6	2.8 - 14.1
3600	5	9	4.8	2.9 - 8.5
4800	3	4	3.7	3.2 - 9.1

* WBC X 10³/μl

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FLAVONE ACETIC ACID
HEMATOLOGIC TOXICITY

DOSE LEVEL (MG/M2)	NUMBER PATIENTS	NUMBER COURSES	MEDIAN PLATELET* NADIR	PLATELET RANGE
100	3	5	221	157 - 789
400	5	18	219	161 - 545
1000	8	21	249	179 - 847
1500	5	10	279	200 - 624
2000	5	7	345	162 - 432
2700	4	9	175	151 - 333
3600	5	9	195	166 - 313
4800	3	4	238	232 - 303

* PLATELET COUNT X $10^3/\mu l$

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FLAVONE ACETIC ACID
HONNEMATOLOGIC TOXICITY

EPISODES TOXICITY (MEDIAN GRADE)

DOSE (MG/M2)	NUMBER COURSES	GASTRO- INTESTINAL	FATIGUE	SEDATION	IV SITE BURNING
100	5	1 (2)	-	-	-
400	18	5 (1)	5 (1)	3 (1)	-
1000	19	4 (1)	4 (1)	5 (1)	-
1500	10	1 (1)	3 (1)	-	1 (1)
2000	7	1 (1)	3 (1)	1 (1)	2 (1)
2700	9	5 (1)	3 (1)	-	8 (1)
3600	9	6 (1)	1 (1)	-	5 (1)
4800	4	1 (1)	1 (2)	-	4 (1)

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SUMMARY

FLAVONE ACETIC ACID

**EIGHTY-THREE COURSES OF FLAVONE ACETIC
ACID HAVE BEEN ADMINISTERED TO 31 PATIENTS;
NO SIGNIFICANT TOXICITY HAS BEEN OBSERVED.
DOSE LEVEL 8 (4800 MG/M2) HAS BEEN COMPLETED
AND WE ARE READY TO PROCEED WITH LEVEL 9
(6400 MG/M2). DOSE ESCALATION WILL CONTINUE
AS PLANNED UNTIL AN MTD IS ACHIEVED.**

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FLAVONE ACETIC ACID

Pharmacology Report

The Ohio State University

INTRODUCTION

Clinical pharmacokinetic studies have continued to be conducted in parallel with the Phase I evaluation of flavone acetic acid (NSC 347512, FAA). The present report summarizes the plasma pharmacokinetics in 26 patients following a one to three hour infusion of the drug on the single schedule. Previous reports have described the renal excretion in 9 of the patients, the analytical method and studies to characterize the FAA metabolites.

METHODS

1. Analytical Method

The reversed-phase HPLC method which was developed for the quantitation of plasma and urine levels of FAA was presented in detail at the Phase I Working Group meeting of July, 1986.

2. Clinical Pharmacokinetics

Initially, blood samples were obtained at 15, 30 and 45 minutes during the one hour infusion and at frequent intervals to 30 hours after dosing. As dose escalation occurred, the infusion duration was extended to two hours for the 2700 mg/m² level and to three hours for the 3600 and 4800 mg/m² levels with blood samples collected every 15 minutes during the infusion period and out to 72 hours following the end of the infusion.

During the trial, as doses were escalated, both two and three compartment open models utilizing appropriate infusion equations for the intravenous period (one to three hours) were evaluated for "goodness of fit" to the patient plasma concentration-time data using $1/y^2_{obs}$ as a weighting term. Each patient data set was fitted by the non-linear least squares computer program NONLIN, iterating the microscopic rate constants and V_1 . Plasma concentration-time profiles were generated showing the NONLIN fitted line drawn through the experimentally determined FAA plasma concentrations.

For the doses of 100 through 2000 mg/m², the three compartment open model system provided the best fits. However, as the dose escalation continued, the pharmacokinetics following doses of 2700 mg/m² and higher exhibited two compartment open model behavior and, therefore, were fitted accordingly.

RESULTS AND DISCUSSION

Clinical Pharmacokinetic Studies

The plasma pharmacokinetics are reported for 26 patients treated with 100-4800 mg/m² of FAA.

Plasma Profiles

Representative FAA plasma concentration-time profiles for doses of 2700 and 4800 mg/m² are shown in Figs. 1 and 2. The curves drawn through the experimentally determined plasma concentrations represent the computer fit of the data to a two compartment open model for these dose levels.

Due to the apparent saturable nature of at least one of the elimination pathways of FAA, it was not possible to obtain a satisfactory simultaneous fit of both the infusion and post-infusion phases for the 4800 mg/m² doses. Consequently, a NONLIN fit to only the post-infusion phase was obtained for these doses. The plasma concentration-time profile (Fig. 2) for the 4800 mg/m² dose shows this NONLIN fit superimposed upon the experimental plasma concentrations obtained during the infusion period. Following the end of the infusions, plasma levels generally declined more than 1000-fold during 72 hours.

Apparent Recycling

As reported previously for the lower doses (100-2000 mg/m²), apparent recycling has continued to be observed in the terminal phases of the plasma concentration-time profiles (Figs. 1 and 2). The apparent recycling has not been correlated with the time of food, as would be expected if it were related to enterohepatic recycling, nor has it correlated with urinary pH, as would be expected if it were related to renal tubular reabsorption. The apparent recycling continues to remain unexplained.

Peak Plasma Concentrations

Table 1 shows the mean maximum plasma concentrations of FAA at the four highest dose levels to date. Each peak level was observed immediately prior to the end of the infusion. Table 1 shows that peak levels increased with increasing dose and decreased with increasing infusion duration. Thus, mean peak levels at the 4800 mg/m² dose given over three hours (286.0 µg/ml), were essentially identical to those seen at the 2700 mg/m² dose given over two hours (281.6 µg/ml).

Dose-Dependent Behavior of the Mean Pharmacokinetic Parameters

A summary of the mean pharmacokinetic parameters at each dose level is shown in Tables 2-5. The pharmacokinetic parameters exhibited definite dose-dependent behavior affecting both distribution and elimination.

Areas Under the Plasma Concentration-Time Curves

These changes in disposition were reflected in reductions in the beta and gamma phases areas under the plasma concentration-time curves with corresponding increases in the alpha phase areas as the dose was escalated (Table 2).

The reduction in the beta phase area at the higher doses was so dramatic that the NONLIN program could no longer distinguish the alpha and beta phases, thus preventing the fitting of the plasma concentration-time data to the three compartment open model. Consequently, for doses of 2700 mg/m² and higher, it was necessary to computer fit the data to a two compartment open model. The terminal phase area under the plasma concentration-time curve was somewhat variable, in part due to the apparent recycling, and appeared to be a relatively minor portion of the entire curve, ranging from 10% to 20% at doses of 1000 mg/m² and higher.

Half-Lives

The changes in disposition were also reflected in the changes observed in the disposition half-lives (Table 3). In general, all the harmonic mean disposition half-lives increased with increasing dose, although the terminal half-life appeared to fluctuate randomly between 7 and 23 hours, the random pattern probably reflecting the influence of the recycling. In contrast, the mean residence time (MRT) remained essentially constant, ranging only from 2.5 to 4.6 hours over the entire dosing range.

Apparent Volumes of Distribution

The apparent volumes of distribution also exhibited dose-dependent behavior (Table 4). The central compartment volume of distribution (V_1) showed a trend of increasing with increasing dose, a characteristic consistent with the saturation of plasma protein binding sites. The V_2 volume of distribution of the three compartment open model showed a clear trend of decreasing with increasing dose. The V_3 volume of distribution decreased from 100 to 1000 mg/m² and then appeared to remain constant. The net result on the steady state volume of distribution ($V_{d_{ss}}$) was an initial rapid drop and subsequent slower decline over the entire dose range. The $V_{d_{area}}$ appeared to remain essentially constant over the dose range of 100 to 2000 mg/m². With the limited available data, there is no evident trend in the V_2 volume of distribution nor in $V_{d_{area}}$ for the dose range of 2700 to 4800 mg/m².

Total Body Plasma Clearance

The mean total plasma body clearance decreased from 235.5 to 57.9 ml/min/m² as the dose was increased from 100 to 4800 mg/m² (Table 5). The initial rapid drop in clearance and the subsequent slower decline (Fig. 3) is directly parallel to the behavior observed in $V_{d_{ss}}$ (Fig. 4) and explains the essentially constant MRT ($Cl_T/V_{d_{ss}}$) observed over the entire dose range.

Parallel First-Order and Saturable Elimination Model

The parallel dose-dependent nature in both clearance and $V_{d_{ss}}$ strongly suggests that there is a common saturable mechanism for these parameters. The change in total body plasma clearance (Cl_T) as a function of the FAA dose appears to be characteristic of one or more first-order elimination processes in parallel with one or more saturable Michaelis-Menten elimination processes and can be pharmacokinetically described by the equation in Table 6.

Due to the parallel first-order components, the individual plasma concentration-time profiles appear to exhibit pseudo-first-order decay.

Mean Cl_T values for each dose level were NONLIN fitted with weights of $1/y^2_{obs}$ to the equation shown in Table 6. An excellent fit to the equation was obtained (Fig. 3) and provided the parameter estimates shown in Table 7. Note in particular that the Michaelis constant, corresponding to the dose which would cause a mean 50% saturation of the Michaelis-Menten processes, is 312 mg/m². This Michaelis constant and the V_{max} term are equivalent to the classical Michaelis-Menten parameters times a volume of distribution term ($2 \times Vd_{ss}$).

The steady state volume of distribution, Vd_{ss} , was similarly fitted (Fig. 4) to an analogous equation shown in Table 8. The Michaelis constant from this fit of Vd_{ss} is 295 mg/m², a value essentially identical to that obtained from the clearance fit.

This identity of the Michaelis constants further supports the concept that Cl_T and Vd_{ss} share a common saturable pathway within the same organ having both elimination and tissue binding functions. The rate-limiting step for both drug elimination and drug-tissue binding within such an organ could be a capacity-limited tissue uptake mechanism.

From the NONLIN fits of Cl_T and Vd_{ss} as a function of dose, it is projected that, as the dose approaches infinity, Cl_T will approach a mean value of 36.1 ml/min/m² and Vd_{ss} will approach a mean value of 5.82 L/m². The Vd_{ss} term may be further complicated by the increasing degree of saturation of plasma protein binding.

All other dose-dependent pharmacokinetic parameters appear to be secondary factors to the dose-dependent behavior observed in Cl_T and Vd_{ss} .

Based upon the NONLIN fitted parameters obtained from the fits of Cl_T and Vd_{ss} over the dose range of 100 to 4800 mg/m², the projected mean Cl_T and Vd_{ss} for the next two dose levels are shown in Table 9. A mean Cl_T of 48.3 ml/min/m² is estimated for the 6400 mg/m² level while a mean Cl_T of 45.4 ml/min/m² is estimated for the 8500 mg/m² level. For these respective doses, the additional reductions in Cl_T will result in 17% and 22% greater than expected areas under the plasma concentration-time curves had clearance remained constant and followed purely first-order behavior.

PHARMACOKINETIC SUMMARY

1. Flavone acetic acid was eliminated by a pseudo-first-order process and the plasma levels exhibited apparent tri-exponential decay within the dose range 100 to 2000 mg/m².

Computer fits of the plasma levels for this dose range to a three compartment open model were obtained using NONLIN.

2. A shift in the mean percentage of the area under the plasma concentration-time curve from the beta and gamma phases to the alpha phase was observed with an increase in dose from 100 to 2000 mg/m².

At doses of 2700 mg/m² and higher, the plasma profiles exhibited bi-exponential decay and the plasma levels were fitted to a two compartment open model.

3. The dose-dependent pharmacokinetics was demonstrated by a decrease in the mean total body plasma clearance from 235.5 to 57.9 ml/min/m² as the dose was increased from 100 to 4800 mg/m².
4. In general, prolongation of the exponential half-lives with increasing dose was observed corresponding to the reduction in clearance.
5. For the dose range 2700 to 4800 mg/m², no apparent further shift in the mean percentage of the area corresponding to each of the phases under the plasma concentration-time curve was observed with increase in dose.
6. The mean steady state volume of distribution was observed to decrease with an increase in dose from 100 to 2000 mg/m² ($V_{d_{ss}}$ from 49.24 L/m² to 19.30 L/m²).

A further small decline in $V_{d_{ss}}$ was observed as the dose was escalated to 4800 mg/m².

7. The mean central compartment volume of distribution was observed to increase with increasing dose from 100 to 2000 mg/m² (V_1 from 3.48 L/m² to 6.32 L/m²).

With a further increase in dose to 2700 mg/m², V_1 and V_2 of the three compartment model appeared to merge to yield a single compartment V_1 .

With increase in dose from 2700 to 4800 mg/m², V_1 was observed to increase from 5.05 to 9.98 L/m².

8. The total body volume of distribution, $V_{d_{area}}$, was apparently not dependent upon dose from 100 to 2000 mg/m² suggesting the presence of a small tissue compartment with extremely tight binding.

The reduction in Cl_T with increasing dose and the apparent independence of $V_{d_{area}}$ with dose results in a prolongation of the terminal half-life with increasing dose in this dose range.

At doses of 2700 mg/m² and above, insufficient data prevents evaluation of the trend in $V_{d_{area}}$.

9. The decrease of both $V_{d_{ss}}$ and Cl_T with increasing dose is consistent with a capacity-limited tissue uptake mechanism.

Capacity-limited tissue uptake is reflected in a shift in the areas corresponding to the beta and gamma phases of the AUC to the alpha phase for the lower doses.

10. Both Cl_T and Vd_{ss} were NONLIN fitted as a function of dose employing Michaelis-Menten equations with parallel first-order components for the dose range 100 to 4800 mg/m².

Projection from these fits would predict that the mean Cl_T will approach a value of 36.1 ml/min/m² and the mean Vd_{ss} will approach a value of 5.82 L/m² as dose escalation continues.

11. Predictions for Cl_T at doses of 6400 and 8500 mg/m² are 48.3 and 45.4 ml/min/m², respectively.

Therefore, plasma levels are predicted to be approximately 17 and 22% higher than would be expected from the 33% dose escalations with linear pharmacokinetics.

12. Variation in the plasma levels of FAA particularly in the terminal phase of the plasma profiles, suggestive of a recycling phenomena, was observed throughout the dose range.

TABLE 1

OBSERVED MEAN MAXIMUM PLASMA CONCENTRATIONS
OF FAA IN PATIENTS FOLLOWING IV INFUSION

Dose (mg/m ²)	Patients (n)	Infusion Duration (hr)	Obs. Cp _{max} (μg/ml)
2000	4	1	217.4
2700	2	2	281.6
3600	2	3	229.1
4800	2	3	286.0

TABLE 2

MEAN PERCENTAGE OF THE AREA UNDER THE FLAVONE
ACETIC ACID PLASMA CONCENTRATION-TIME CURVE
CORRESPONDING TO EACH OF THE DECAY PHASES
AT EACH DOSE LEVEL FOLLOWING ONE TO THREE
HOUR INFUSION

Dose (mg/m ²)	Patients (n)	Alpha Phase %	Beta Phase %	Gamma Phase %
100	3	33.15	36.62	30.22
400	4	31.48	42.74	25.79
1000	6	55.23	36.82	7.97
1500	3	63.04	29.35	7.61
2000	4	74.13	15.84	10.03
		Alpha Phase %	Beta Phase %	
2700	2	91.66	8.34	
3600	2	81.20	18.80	
4800	2	87.50	12.50	

TABLE 3

FLAVONE ACETIC ACID HARMONIC MEAN HALF-LIVES
AND MEAN RESIDENCE TIMES IN PATIENTS FOLLOWING
ONE TO THREE HOUR IV INFUSION

Dose (mg/m ²)	Patients (n)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (hr)	$t_{1/2\gamma}$ (hr)	MRT (hr)
100	3	3.12	0.67	6.98	3.19
400	4	5.25	0.82	8.96	3.73
1000	6	18.88	0.62	13.65	2.82
1500	3	12.96	1.41	22.56	2.53
2000	4	44.97	2.34	18.30	4.20
		$t_{1/2\alpha}$ (min)		$t_{1/2\beta}$ (hr)	
2700	2	59.90		9.97	2.57
3600	2	66.70		8.03	3.11
4800	2	101.43		14.43	4.61

TABLE 4

FLAVONE ACETIC ACID MEAN VOLUME PHARMACOKINETIC
PARAMETERS

Dose (mg/m ²)	Patients (n)	V ₁ (L/m ²)	V ₂ (L/m ²)	V ₃ (L/m ²)	Vd _{ss} (L/m ²)	Vd _{area} (L/m ²)
100	3	3.48	7.05	38.71	49.24	160.18
400	4	3.68	5.24	26.32	35.24	122.44
1000	6	4.84	4.60	11.28	20.71	163.59
1500	3	4.17	2.59	10.06	16.81	158.36
2000	4	6.32	1.75	11.23	19.30	127.66
		V ₁ (L/m ²)	V ₂ (L/m ²)			
2700	2	5.05	3.12	8.16	45.88	
3600	2	6.71	4.65	11.35	41.44	
4800	2	9.98	6.66	16.64	72.52	

TABLE 5

VARIATION OF TOTAL BODY CLEARANCE OF
FAA IN PATIENTS FOLLOWING ONE TO
THREE HOUR IV INFUSION

Dose (mg/m ²)	Patients (n)	Mean Cl _T (ml/min/m ²)	S.D. (ml/min/m ²)
100	3	235.5	126.5
400	4	149.3	61.4
1000	6	107.5	20.8
1500	3	80.9	23.1
2000	4	77.3	35.0
2700	2	53.5	.
3600	2	58.0	.
4800	2	57.9	.

TABLE 6

PARALLEL FIRST-ORDER AND SATURABLE
MICHAELIS-MENTEN ELIMINATION PATHWAYS

$$Cl_T = \frac{V_{max'}}{K_m' + Dose} + Cl'$$

$V_{max'}$ = Sum of all maximum velocity terms

K_m' = Michaelis constant term

Cl' = Sum of all first-order elimination
processes

TABLE 7
NONLIN FIT OF TOTAL BODY CLEARANCE vs DOSE

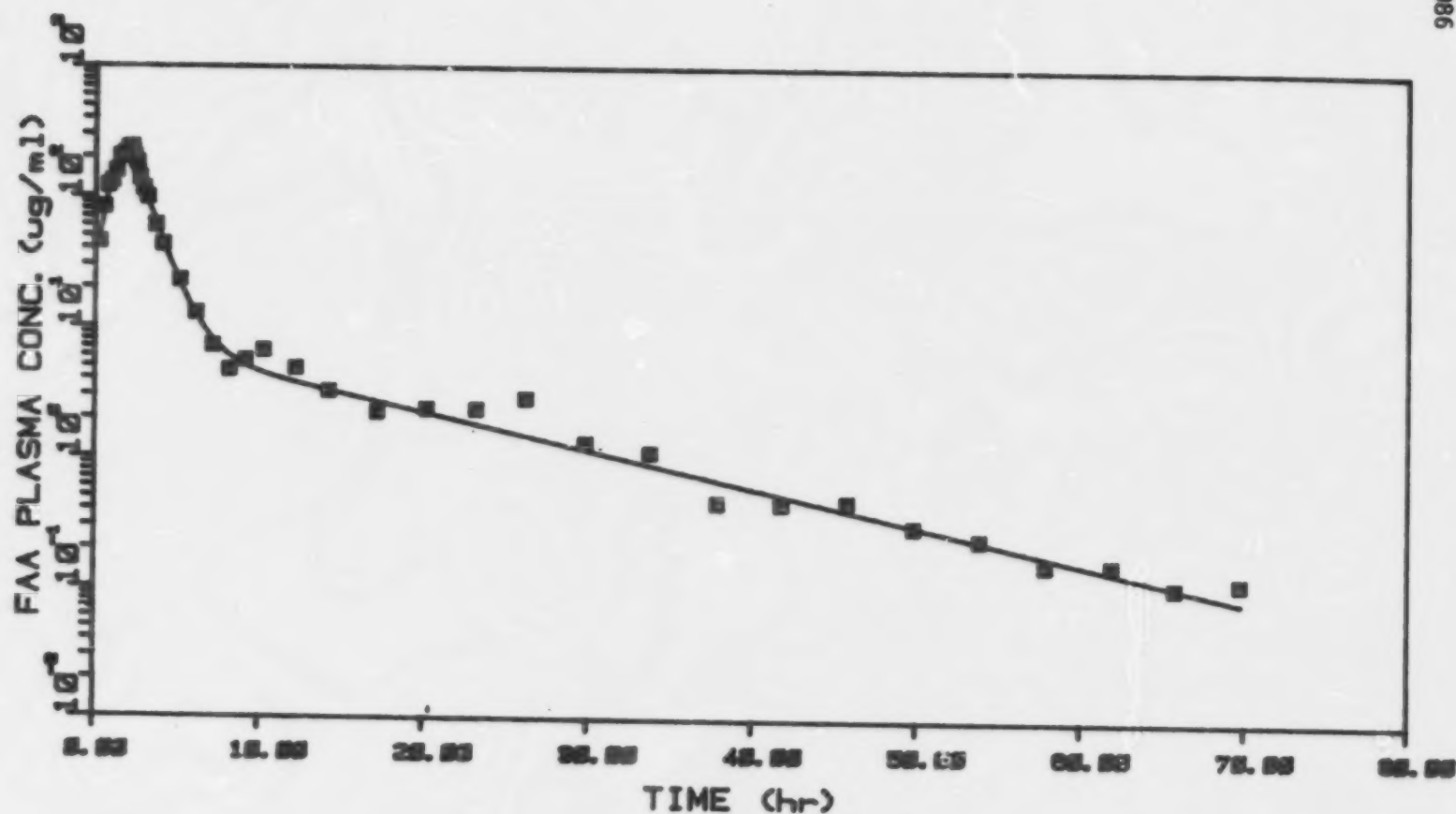
$$\begin{aligned} V_{\max}' &= 82,421 \text{ mg}\cdot\text{ml}/\text{min}/\text{m}^4 \\ K_m' &= 312 \text{ mg}/\text{m}^2 \\ Cl' &= 36.1 \text{ ml}/\text{min}/\text{m}^2 \\ Cl_T^{\max} &= \frac{V_{\max}'}{K_m'} + Cl' = 300.3 \text{ ml}/\text{min}/\text{m}^2 \end{aligned}$$

TABLE 8
DOSE-DEPENDENT BEHAVIOR OF THE STEADY
STATE VOLUME OF DISTRIBUTION

$$\begin{aligned} V_{d_{ss}} &= \frac{V_{\max}''}{K_m'' + \text{Dose}} + V_{d_{ss}}'' \\ V_{d_{ss}}^{\max} &= 67.05 \text{ L}/\text{m}^2 \\ V_{d_{ss}}^{\min} &= 5.82 \text{ L}/\text{m}^2 \\ K_m'' &= 295 \text{ mg}/\text{m}^2 \end{aligned}$$

TABLE 9
PREDICTIONS FOR THE NEXT DOSE LEVELS

Dose (mg/m ²)	Cl _T (ml/min/m ²)	V _{d_{ss}} (L/m ²)
6400	48.3	8.52
8500	45.4	7.88

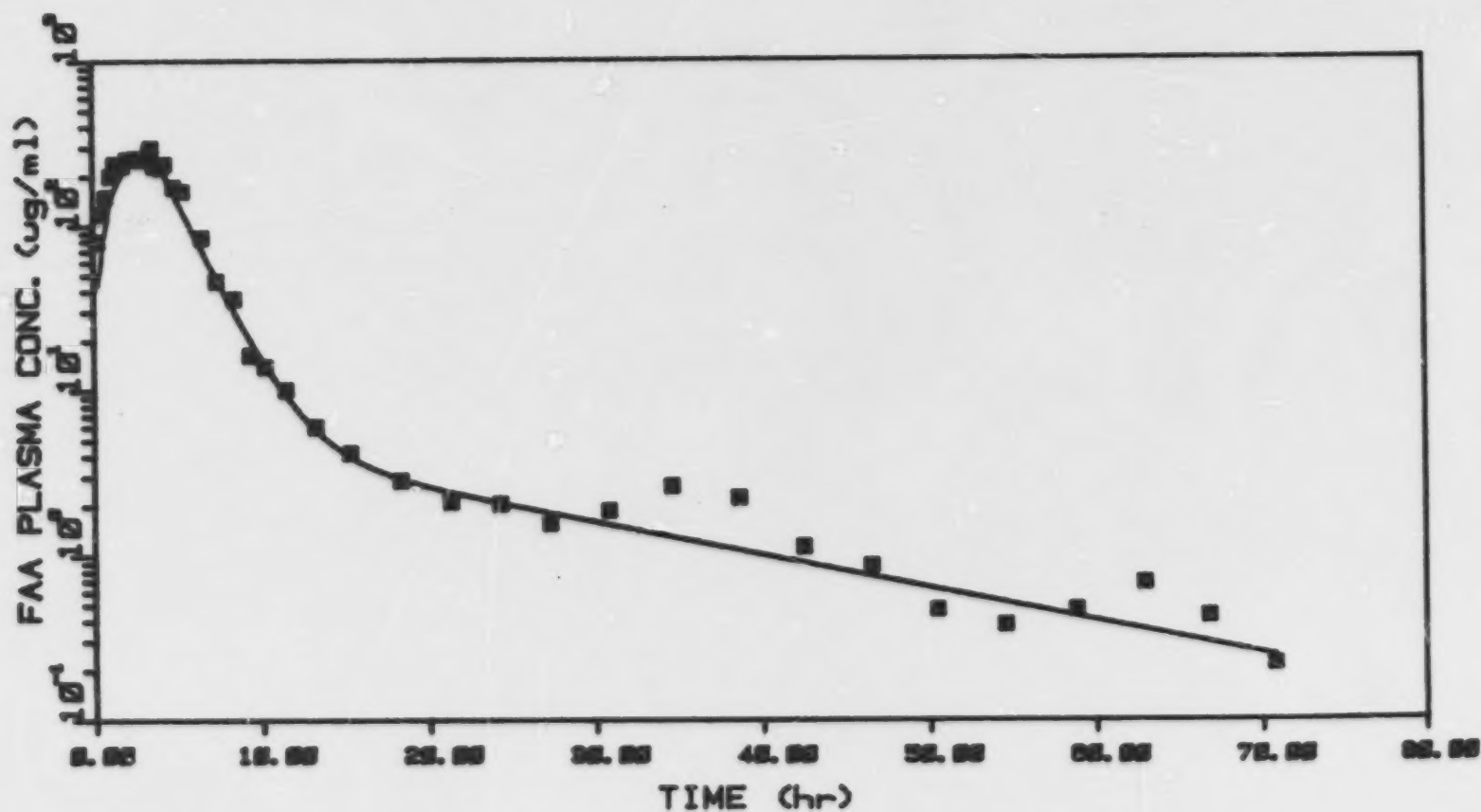


PATIENT D. B.
DOSE = 2700 MG/METER SQ. FAA
CLEARANCE = 69.36 ML/MIN/METER SQ.

Fig. 1

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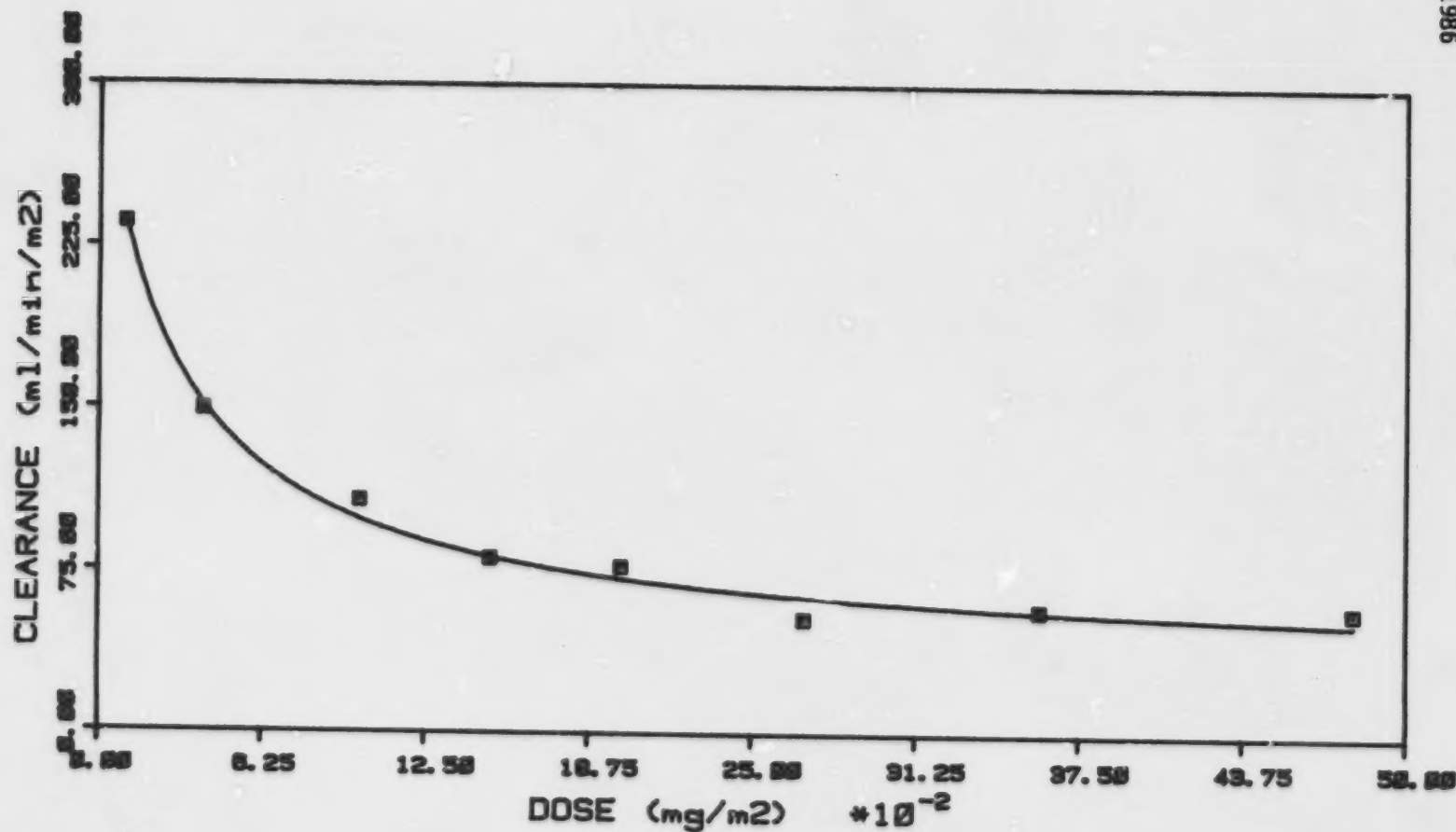
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PATIENT J. S.
 DOSE = 4800 MG/METER SQ. FAA
 CLEARANCE = 58.03 ML/MIN/METER SQ.

Fig. 2

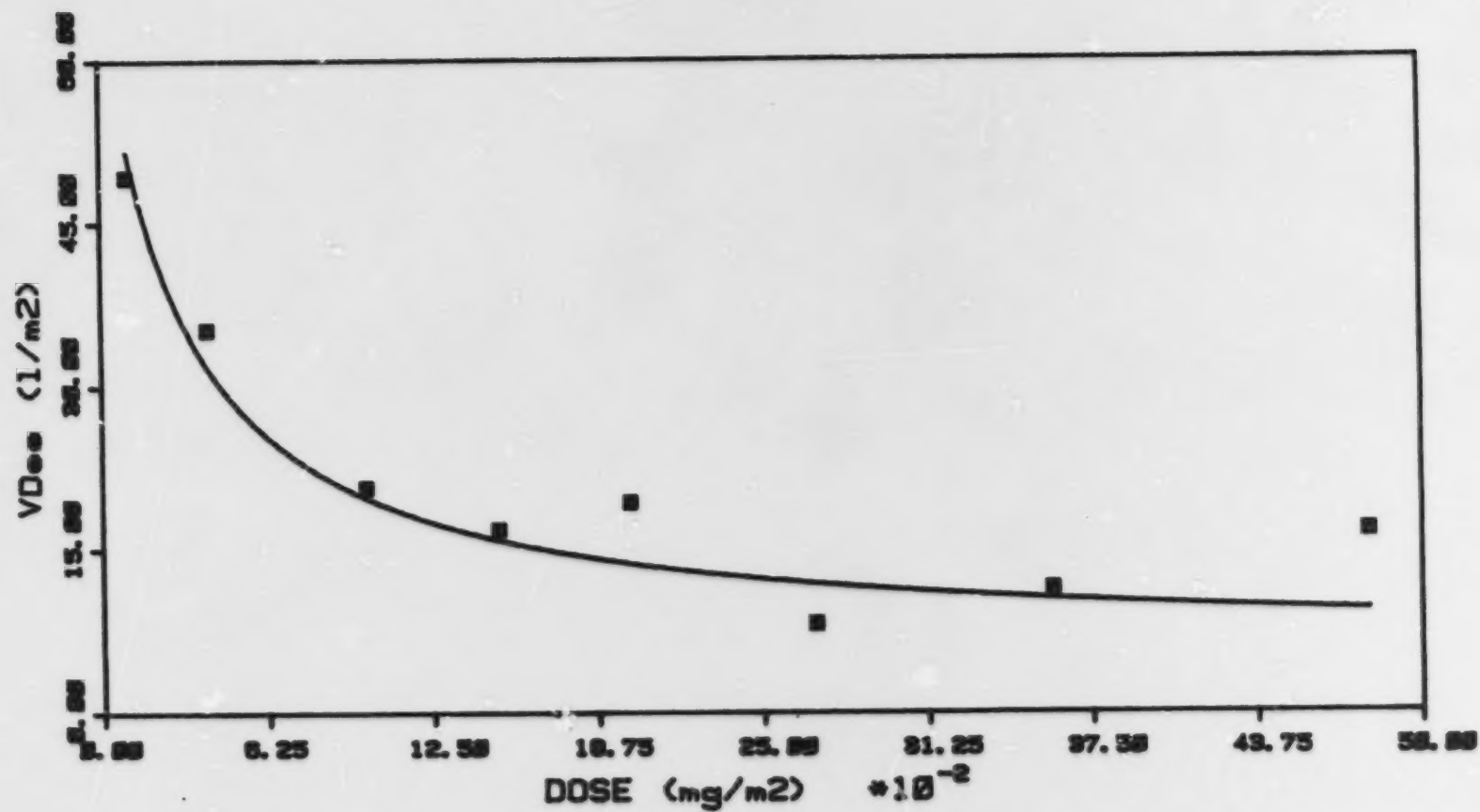
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FLAVONE ACETIC ACID
MAXIMUM MEAN CLEARANCE = 300.26 ML/MIN/M2
MINIMUM MEAN CLEARANCE = 36.05 ML/MIN/M2

Fig. 3

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FLAVONE ACETIC ACID
 MAXIMUM MEAN V_{dss} = 67.05 L/M²
 MINIMUM MEAN V_{dss} = 5.82 L/M²

Fig. 4

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UMCC PHASE I FAA

SCHEDULE:	SINGLE DOSE, Q 3 WEEKS
ADMINISTRATION:	DRUG SOLUTION QS TO 500 cc WITH NS, IV OVER 1 HOUR
HYDRATION/ ALKALINIZATION:	IV FLUIDS WITH 2 AMPS SODIUM BICARBONATE PER LITER, URINARY PH $\geq$ 7 PRE & POST- TREATMENT X 48 HOURS
ESCALATION SCHEME:	1.0 $\rightarrow$ 1.5 $\rightarrow$ 2.0 $\rightarrow$ 2.7 $\rightarrow$ 3.6 4.8 $\rightarrow$ 6.4 GM/M ²

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**UMCC PHASE I FAA
PATIENT CHARACTERISTICS**

NO. PATIENTS/NO. COURSES	22/32
FEMALE/MALE	7/15
PERFORMANCE STATUS	
MEDIAN	1
RANGE	0-2
AGE (YEARS)	
MEDIAN	65
RANGE	34-76
PRIOR THERAPY	
NO PRIOR THERAPY	2
CHEMO	11
CHEMO + RT	8
RADIATION	1

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**UMCC PHASE I FAA
TUMOR TYPES**

COLON	8
LUNG (NSC)	4
MELANOMA	2
RENAL	2
UNKNOWN 1 ^o	1
SARCOMA (OSTEO)	1
PROSTATE	1
ESOPHAGEAL	1
COLON + ESOPHAGEAL	1
LUNG + PROSTATE	1

NO MYELOSUPPRESSIVE EFFECTS

**UMCC PHASE I FAA
NON-HEMATOLOGIC TOXICITY**

**HOT FLUSHES
GASTROINTESTINAL
CARDIAC
CNS**

UMCC PHASE I FAA
HOT FLUSHES

DOSE GM/M ²	NO. PTS./ NO. COURSES	NUMBER COURSES (%)
1.0	3/5	0
1.5	3/5	0
2.0	3/5	0
2.7	3/5	1 (20)
3.6	3/3	3 (100)
4.8	5/7	7 (100)
6.4	2/2	2 (100)

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UMCC PHASE I FAA
GASTROINTESTINAL TOXICITY
(NO. COURSES WITH TOXICITY)

DOSE GM/M ²	NO. PTS/ NO. COURSES	NAUSEA	VOMITING	DIARRHEA	ABNORMAL TASTE
1.0	3/5	0	0	0	0
1.5	3/5	0	0	2	0
2.0	3/5	2	1	0	0
2.7	3/5	0	0	0	0
3.6	3/3	0	1	0	1
4.8	5/7	6	5	5*	1
6.4	2/2	1	1	0	0

* 1 Patient with colon/esophagel carcinoma developed hematochezia 1 hr. after FAA.
sigmoidoscopy 3 days later - negative.

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**UMCC PHASE I FAA
CNS TOXICITY
(NO. COURSES WITH TOXICITY)**

DOSE GM/M ²	1.0	1.5	2.0	2.7	3.6	4.8	6.4
NO. PTS./NO. COURSES	3/5	3/5	3/5	3/5	3/3	5/7	2/2
SEDATION	1		1				
HEADACHE			1			1	
EUPHORIA			1	1			
LIGHT HEADEDNESS			1	1		2	
UPPER EXTREMITY TREMORS						3	
BLURRED VISION						1	1
SEIZURE						1	

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**SEIZURE OBSERVED IN 1 PATIENT
AT 4.8 GM/M² DOSE (3.2 GM)**

**73 YEAR OLD WHITE MALE
DIAGNOSIS: MELANOMA WITH LUNG AND LEFT FOOT METS
PRIOR THERAPY: CHIP X3, DTIC X2
PMH: HYPERTENSION, AODM, ? AORTIC STENOSIS**

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TOXICITIES

DURING INFUSION:

HOT FLUSHES, N/V, UPPER EXTREMITIES TREMORS

**+4 HOUR: DECREASED BP 140/80 - 94/70. NO CHANGE IN
HEART RATE**

**+10 HOUR: VOMITED; UNRESPONSIVE; GENERALIZED
SEIZURE LASTING 30 SECONDS, BP 150/90
K⁺ DECREASED TO 2.8**

NO SEQUELAE

NEGATIVE HEAD CT, EEG, LP

**UMCC PHASE I FAA
CARDIAC TOXICITY
(NO. COURSES WITH TOXICITY)**

DOSE GM/M ²	NO. PTS./ NO. COURSES	<u>EFFECT ON BLOOD PRESSURE</u>		"ANGINA"
		INCREASE ≥ 30 SYSTOLIC	DECREASE ≥ 20 SYSTOLIC	
1.0	3/5	1 (20)	0	0
1.5	3/5	2 (40)	0	0
2.0	3/5	1 (20)	1 (20)	0
2.7	3/5	1 (20)	3 (43)	0
3.6	3/3	1 (20)	0	0
4.8	5/7	2 (29)	3 (43)	0
6.4	2/2	0	2 (100)	1 (50)

**"ANGINA" OBSERVED IN 1 PATIENT
AT 6.4 GM/M² DOSE LEVEL (13.4 GM)**

68 Y.O. WHITE MALE

DX: RENAL CELL CANCER WITH LUNG AND ST METS

PRIOR THERAPY: RIGHT NEPHRECTOMY, NMF X 4 COURSES

**PMH: MANIC DEPRESSION - CONTROLLED
INFERIOR MI BY EKG ONLY**

TOXICITIES

DURING INFUSION:

- +30": PROFUSE DIAPHORESIS, HOT FLUSHES**
- +45": CHEST PRESSURE/TIGHTNESS, NUMBNESS IN JAW**

FAA DISCONTINUED - NO ACUTE EKG OR BP CHANGES

- +50": N/V**
- +1 HOUR: SX RESOLVED WITHOUT MEDICAL TREATMENT**
- +4 HOUR: DECREASED BP 140/84 - 110/76. NO CHANGE
IN HEART RATE**

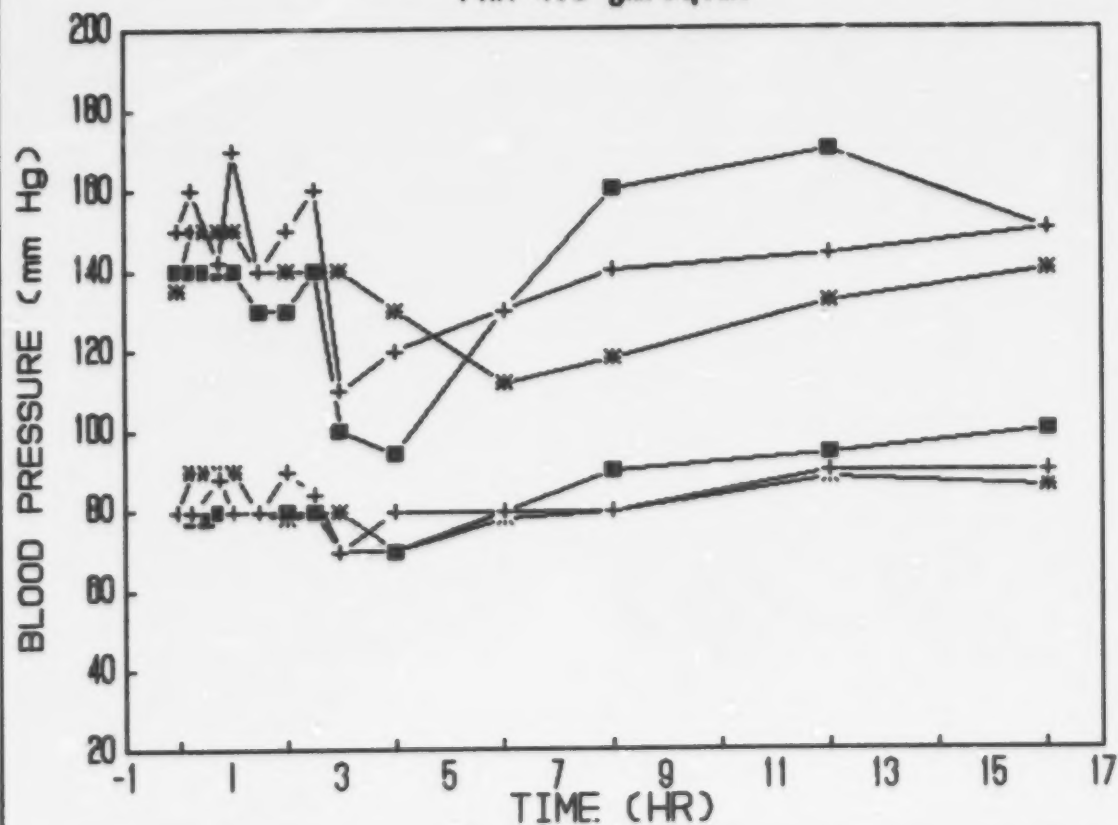
SERIAL EKG'S AND CARDIAC ENZYMES NEGATIVE

**MEDIAN CHANGE IN CARDIOVASCULAR
PARAMETERS FROM PRETREATMENT**

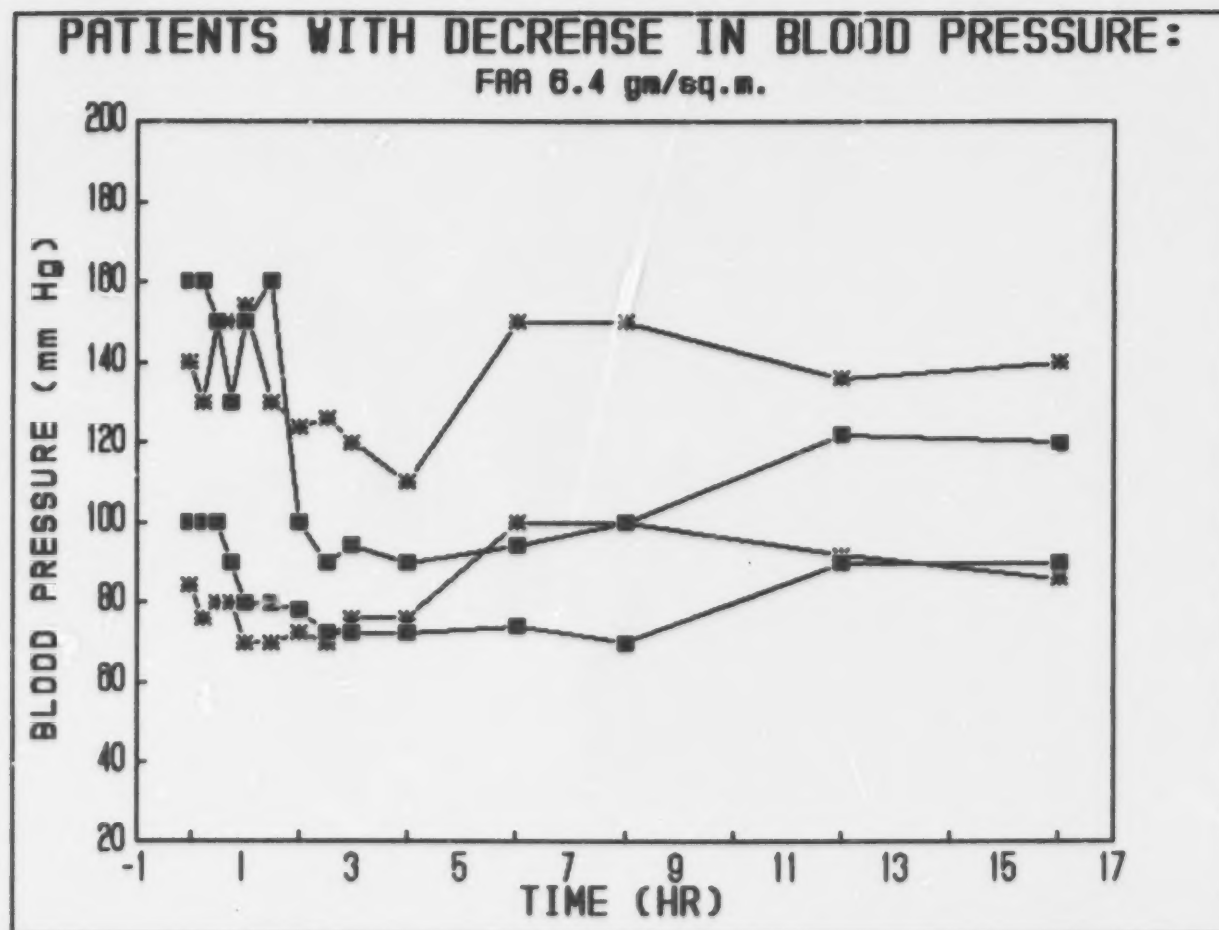
<u>DOSE</u> <u>GM/M²</u>	<u>NO.</u> <u>PTS.</u>	<u>MEDIAN VALUE (RANGE)</u>			<u>TIME</u> <u>HOURS</u>
		<u>DECREASE</u> <u>SYSTOLIC</u> <u>PRESSURE</u> <u>mm Hg</u>	<u>DECREASE</u> <u>DIASTOLIC</u> <u>PRESSURE</u> <u>mm Hg</u>	<u>INCREASE</u> <u>HEART</u> <u>RATE</u> <u>PER/MIN</u>	
2.0	1	30	10	2	6
2.7	3	20 (20-30)	10 (8-20)	10 (0-20)	2.5 (2-3)
4.8	3	40 (23-46)	10 (2-10)	0 (0-13)	4 (3-6)
6.4	2	30, 70	8, 28	0, 16	4, 2.5

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PATIENTS WITH DECREASE IN BLOOD PRESSURE: FAA 4.8 gm/sq.m.



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**MEDIAN PERCENT CHANGE IN CARDIOVASCULAR
PARAMETERS FROM PRETREATMENT**

<u>DOSE</u> <u>GM/M²</u>	<u>NO.</u> <u>PTS.</u>	<u>MEDIAN % (RANGE)</u>			
		<u>DECREASE</u> <u>SYSTOLIC</u> <u>PRESSURE</u>	<u>DECREASE</u> <u>DIASTOLIC</u> <u>PRESSURE</u>	<u>DECREASE</u> <u>MEAN</u> <u>ARTERIAL P.</u>	<u>INCREASE</u> <u>HEART</u> <u>RATE</u>
2.0	1	20	11	14	2
2.7	3	17 (13-17)	17 (11-22)	23 (16-28)	10 (0-20)
4.8	3	27 (17-27)	13 (2-13)	19 (9-22)	0 (0-13)
6.4	2	21, 44	10, 33	15, 36	0, 33

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NO ANTITUMOR ACTIVITY OBSERVED

SUMMARY

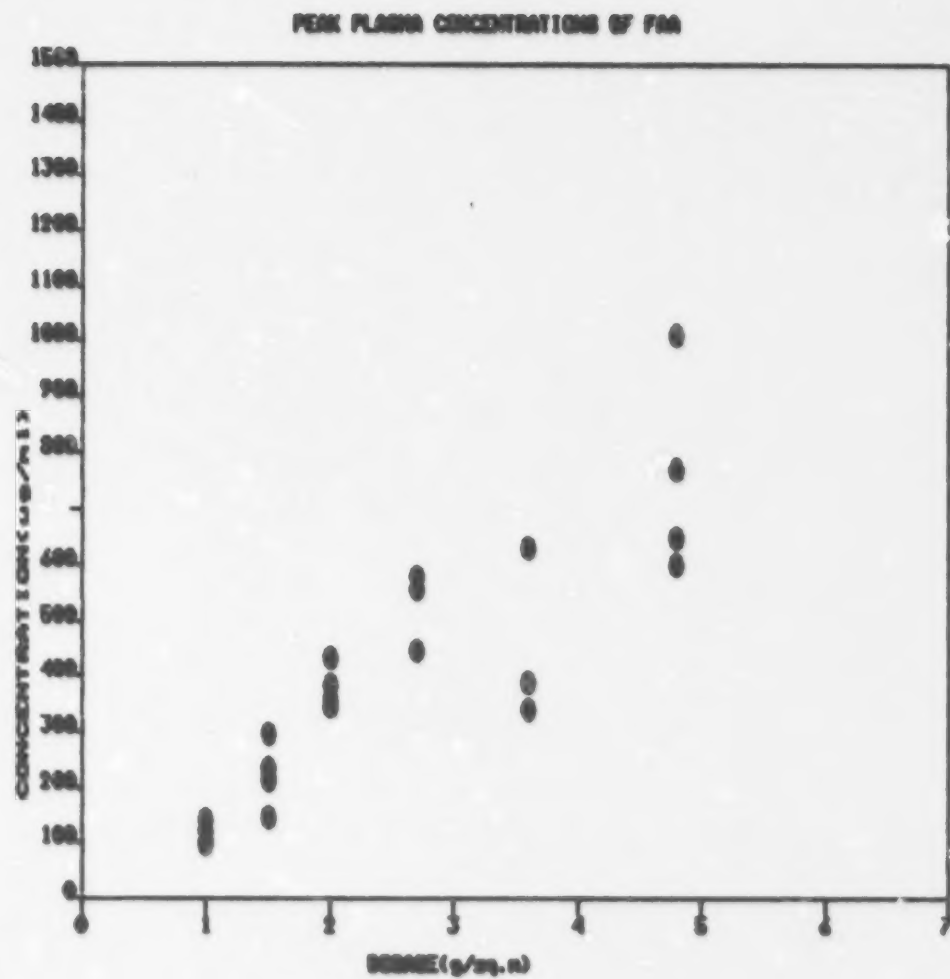
I. TOXICITIES ASSOCIATED WITH FAA ADMINISTRATION INCLUDE:

DOSE RELATED:

1. **GASTROINTESTINAL**
NAUSEA/VOMITING/DIARRHEA - GRADE 1-2
2. **CARDIAC**
DECREASE IN BLOOD PRESSURE - GRADE 1
(MORE FREQUENT AND SEVERE AT THE HIGHER DOSES)
ANGINA (NO ISCHEMIC CHANGES) - GRADE 3
3. **HOT FLUSHES** - MODERATE BUT TOLERABLE

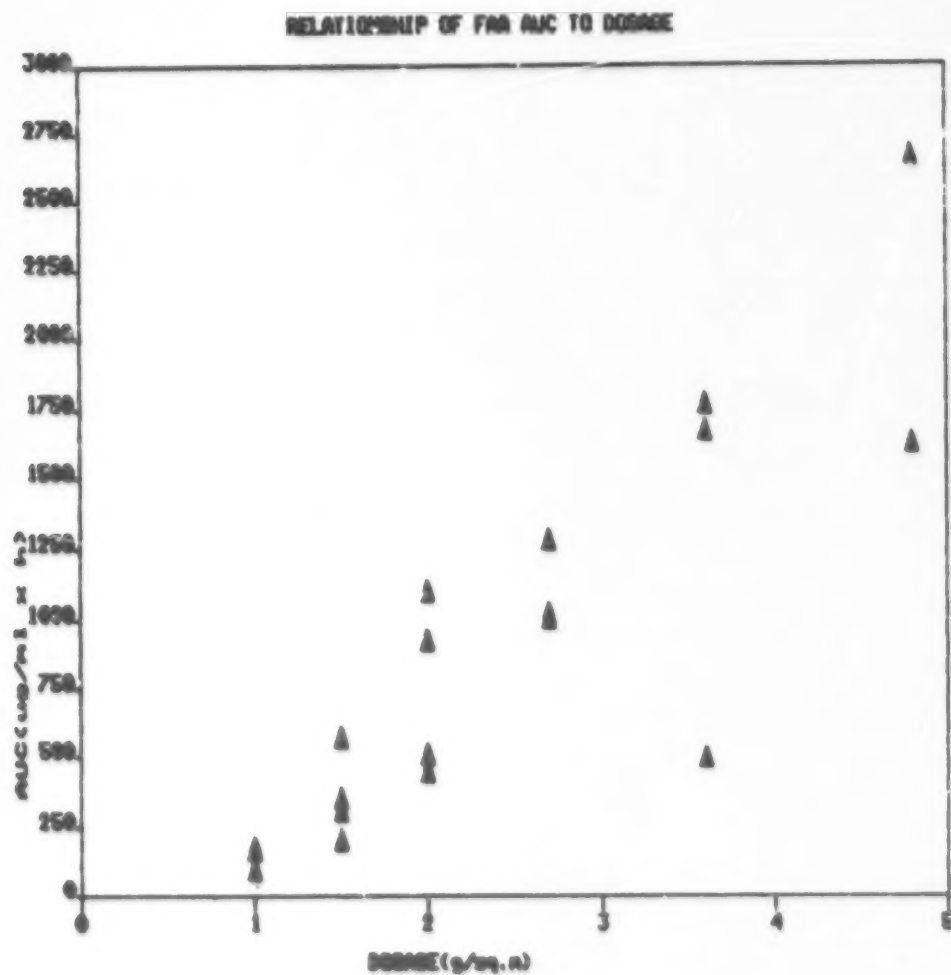
NON-DOSE RELATED

1. **CNS**
SEIZURE - PROBABLY DRUG RELATED
OTHER EFFECTS - SPORADIC, GENERALLY MILD & TRANSIENT
 2. **MODEST BLOOD PRESSURE ELEVATION OBSERVED IN
SOME PATIENTS AT LOWER DOSES ONLY**
- II. THE DOSE-LIMITING TOXICITY WILL MOST LIKELY BE HYPOTENSION.**
- III. THE MTD IS ANTICIPATED TO BE 6.4 GM/M² WITH A RECOMMENDED DOSE
FOR PHASE II TRIALS BEING 4.8 GM/M² ADMINISTERED AS A BOLUS
INFUSION OVER 1 HOUR EVERY 3 WEEKS.**

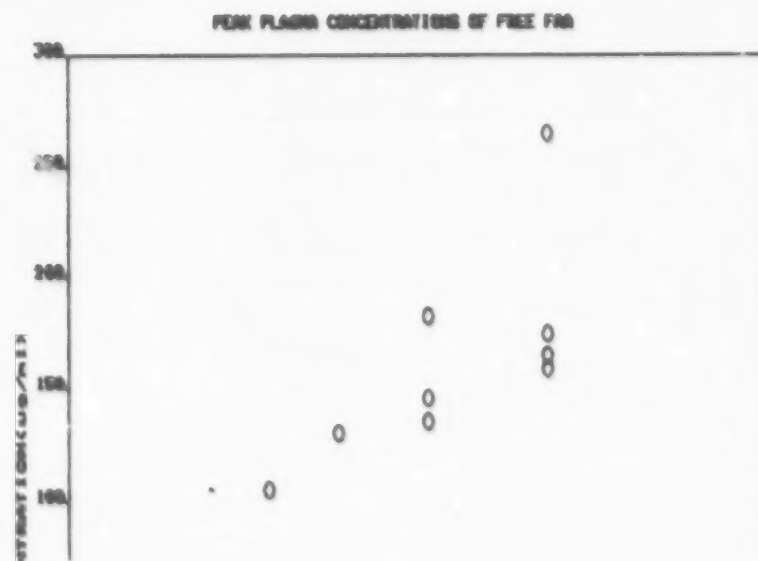
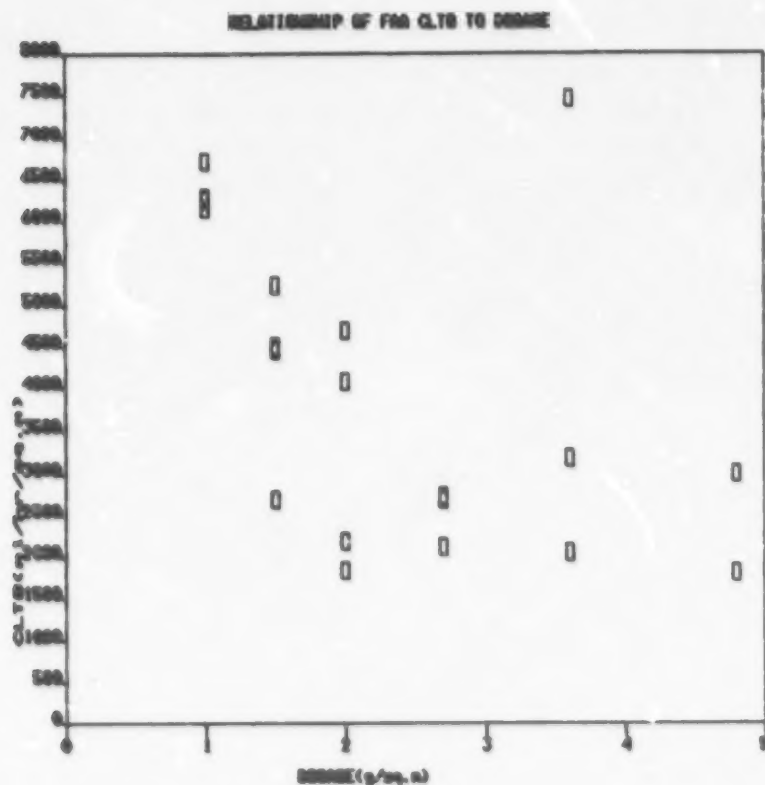


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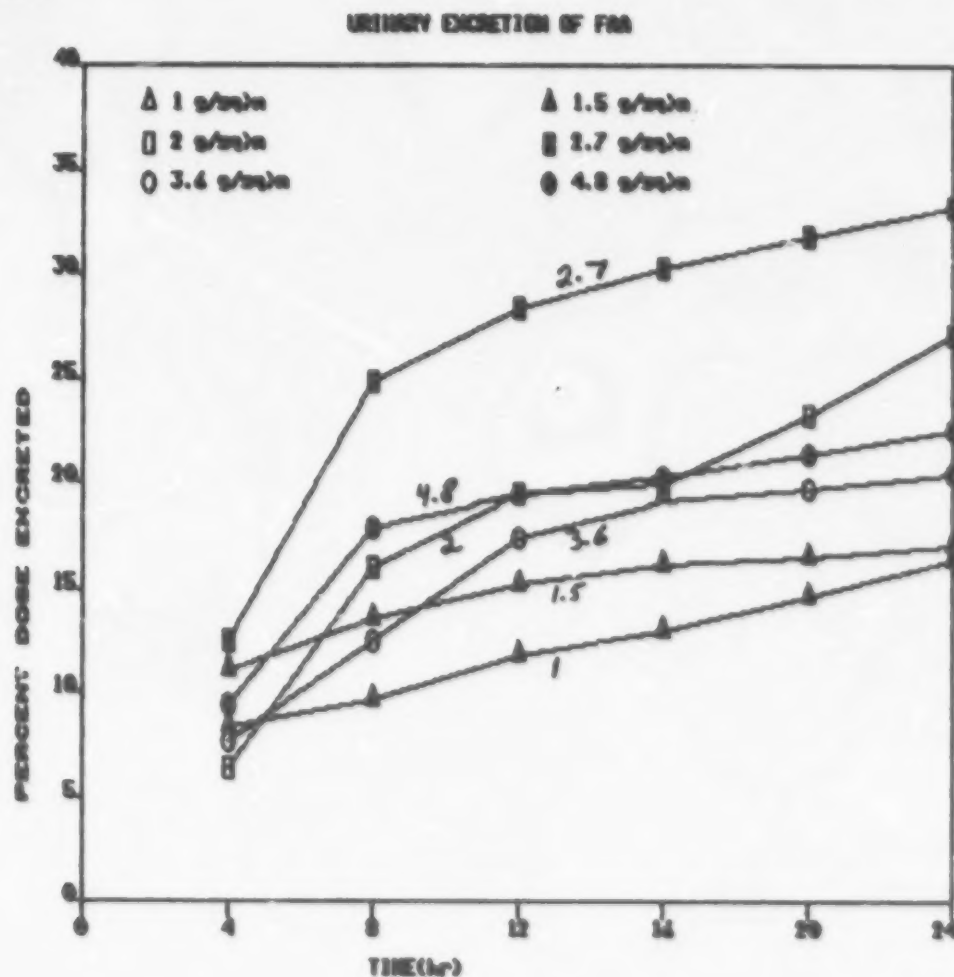
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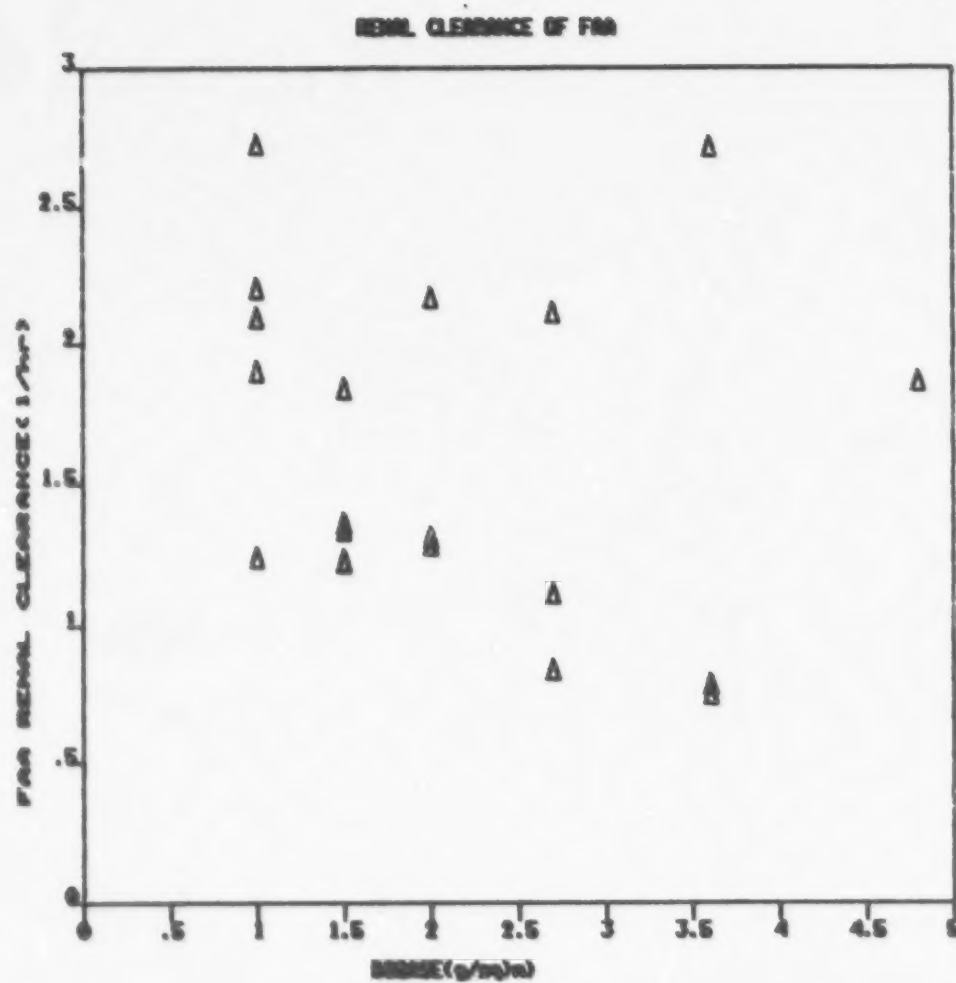
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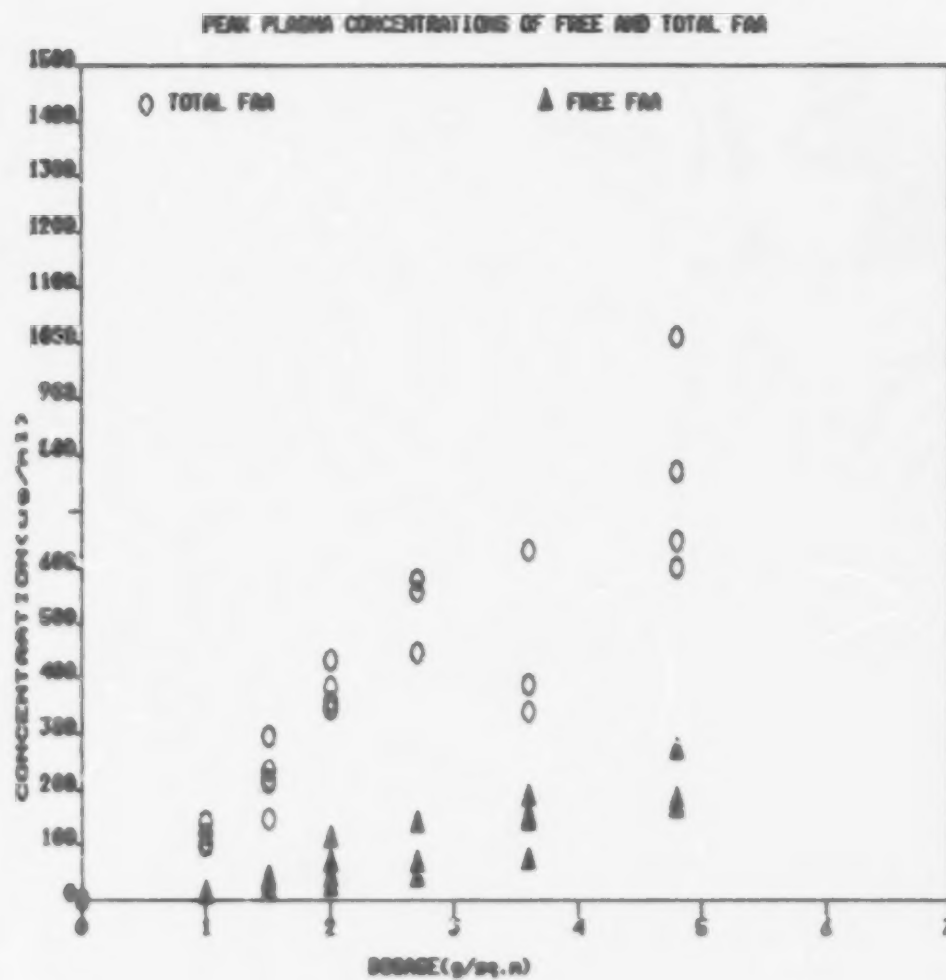


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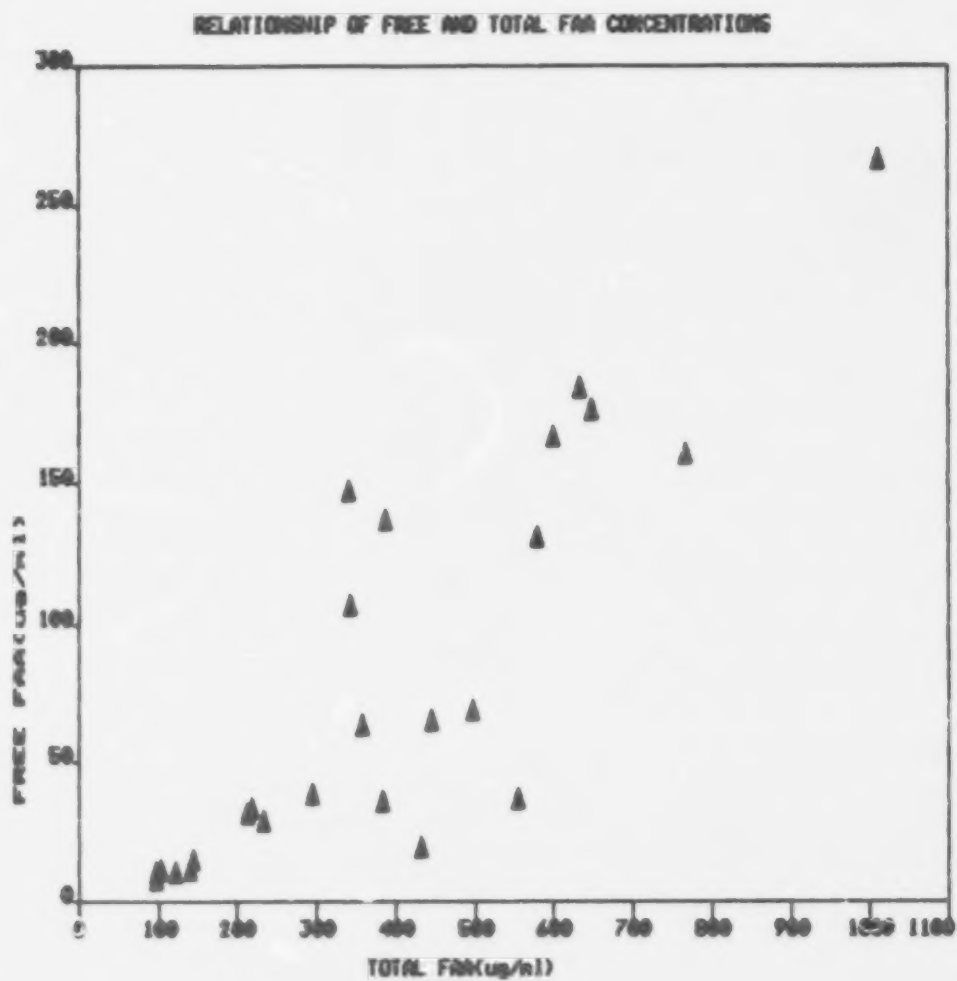


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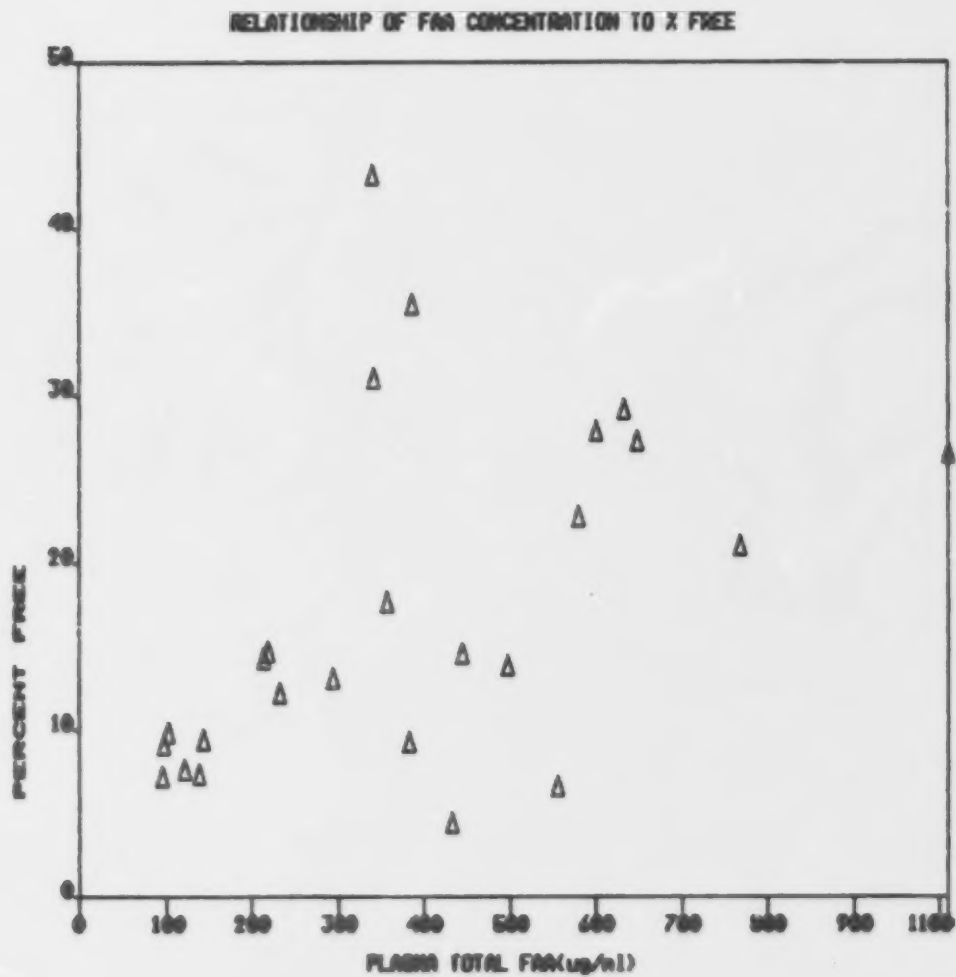


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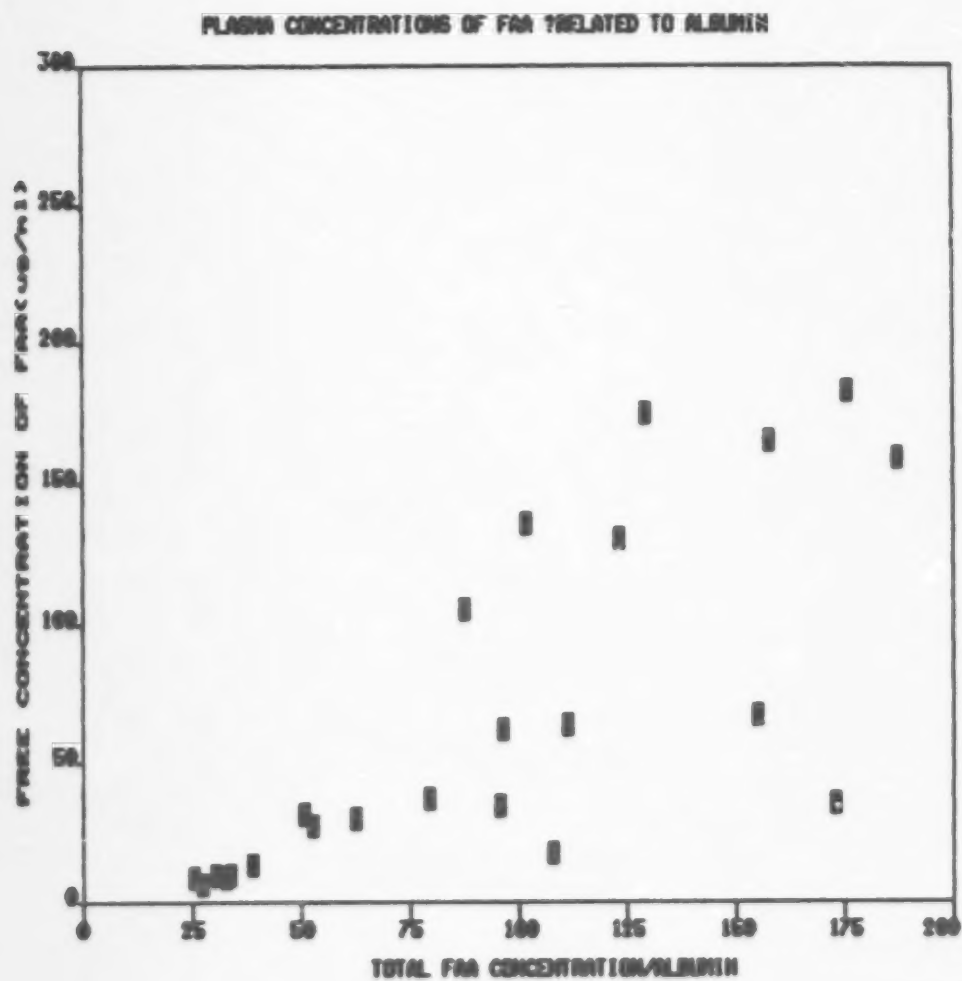


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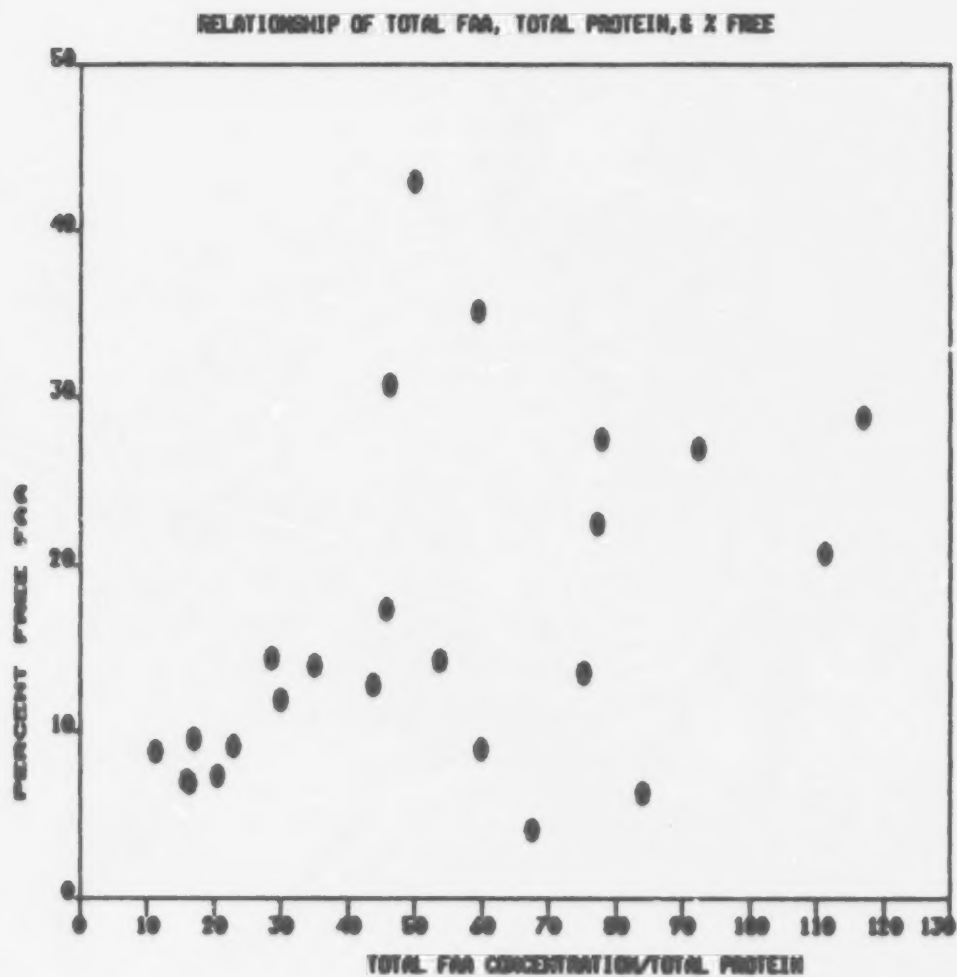
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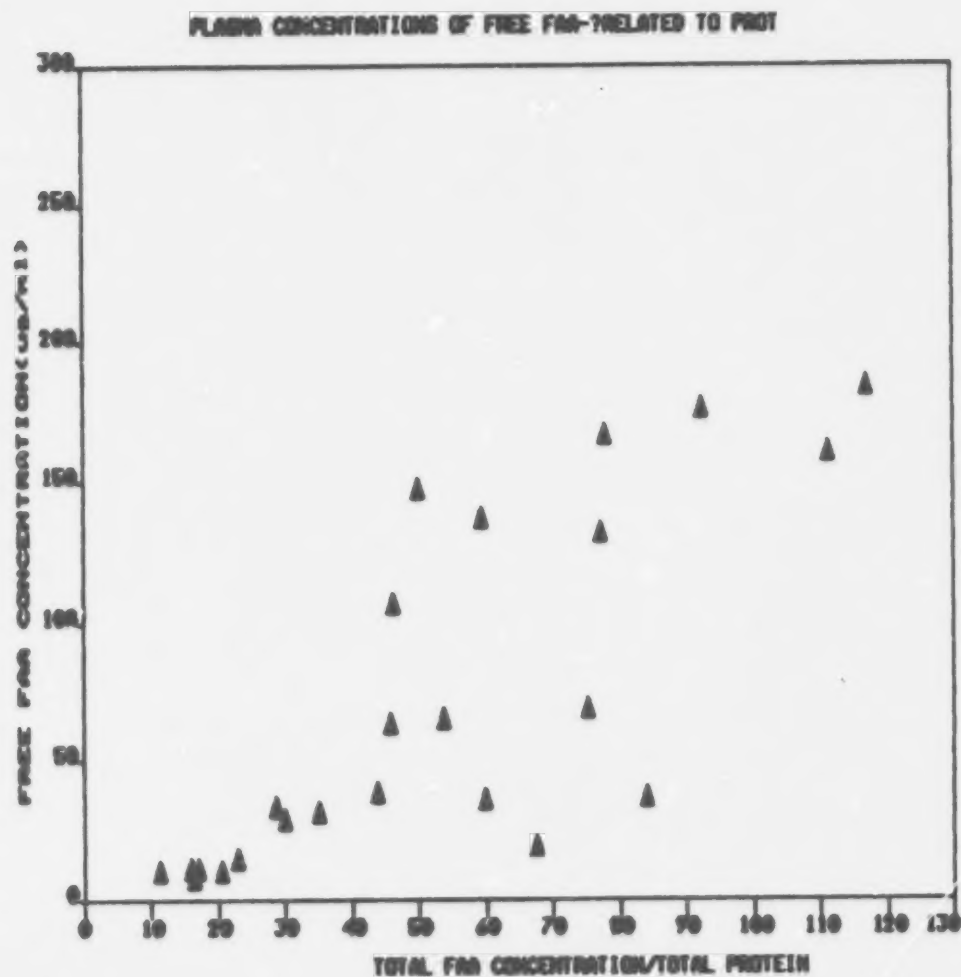
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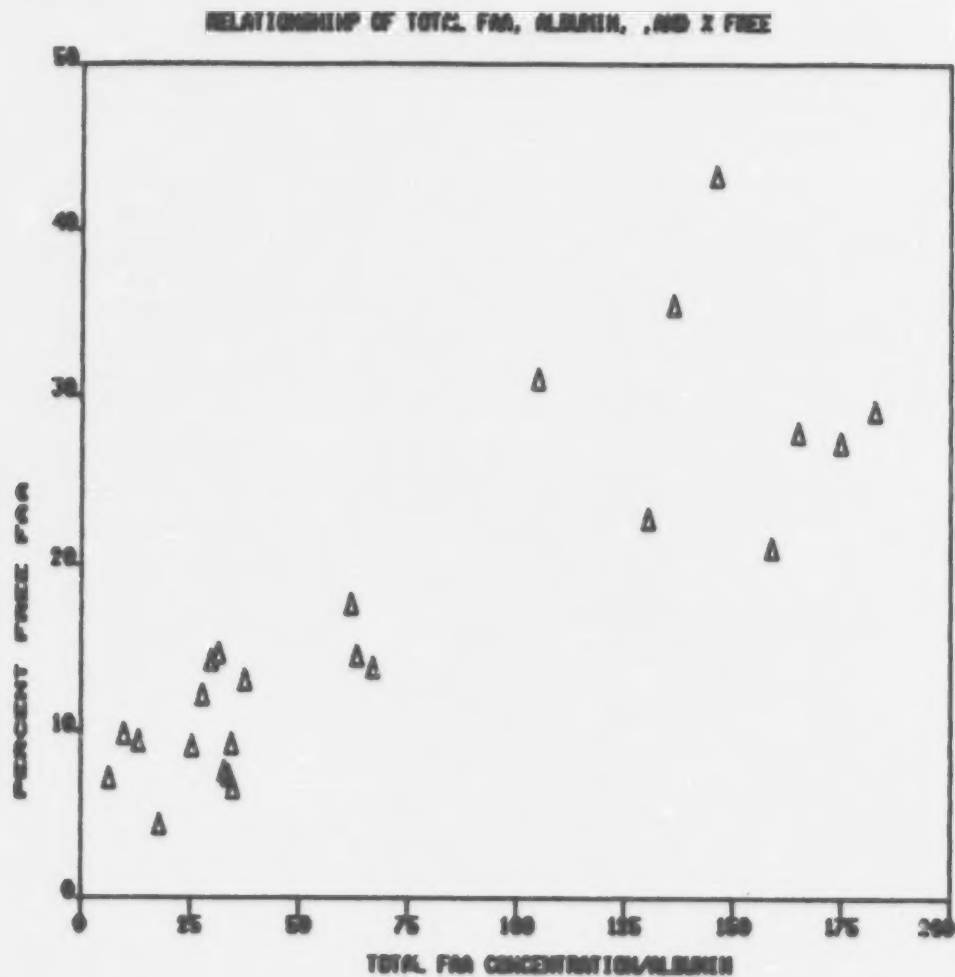
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FAA* --- DOSE ESCALATION SCHEME

<u>DOSE LEVEL</u>	<u>DOSE (mg/sq m)</u>	<u>% INCREASE</u>	<u>No. Patients</u>
Entry	330		3
1st	660	100	3
2nd	1,100	66	3
3rd	1,650	50	3
4th	2,300	35	3
5th	3,000	30	3
6th	3,900	30	3
Additional	+30%	30	3
previous level			

*IV over one hour every week x 4, followed by 2 week rest
Preceded by 1 liter 1/3 normal saline with 100 mEq sodium bicarbonate given over 2 hours prior to FAA to adjust urine pH to greater than pH 7

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PATIENT CHARACTERISTICS

No. of Patients	23
Sex (M/F)	16/7
Age, median (range)	59 (34-76)
Prior Treatment	
None	2
Radiotherapy	8
Chemotherapy	19
Chemo and Radiotherapy	8
Median No. of Previous Drugs	2 (1-3)

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PATIENT DISTRIBUTION BY TUMOR SITE

<u>Primary site</u>	<u>No. Patients</u>
Colon	13
Lung (non-small cell)	5
Ovary	2
Malignant Melanoma	1
Soft Tissue Sarcoma	1
Chordoma	1

COLON CANCER: PATIENT CHARACTERISTICS

(No. of Patients = 13)

PRIOR TREATMENT WITH CHEMOTHERAPY

none	2
with 5-FU alone	8
with 5-FU + other drugs	3

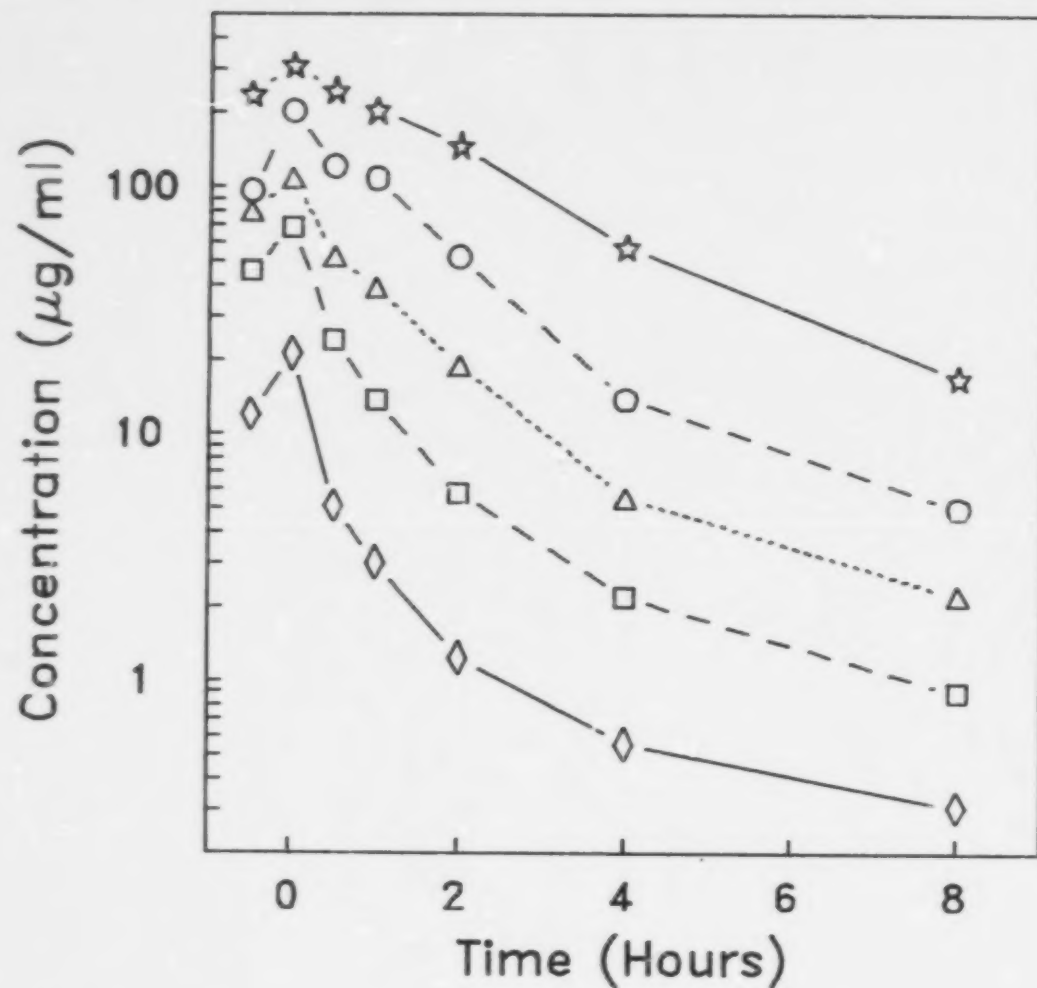
TOXICITY TO DATE (11/86)

- Hypotension (b.p. dec to 80/50) at higher doses
- Hypertension (b.p. inc to 190/86)
- Hypothermia and sweating
- Weakness, somnolence, "warm feeling"
- Nausea, mild and transient
- Watery diarrhea, mild to moderate
- Metallic taste and visual disturbances
- Burning at infusion site

PRELIMINARY CLINICAL OBSERVATIONS

- FAA is well tolerated in weekly IV doses up to 2300 mg/m² over one hour.
- Hypotension occurs at 3,000 and 3,900 mg/m² in one hour in some patients.
- Three hour infusion is being used to avoid hypotension.
- Current dose level is 5,100 mg/m² given over three hours.
- No tumor responses observed so far.

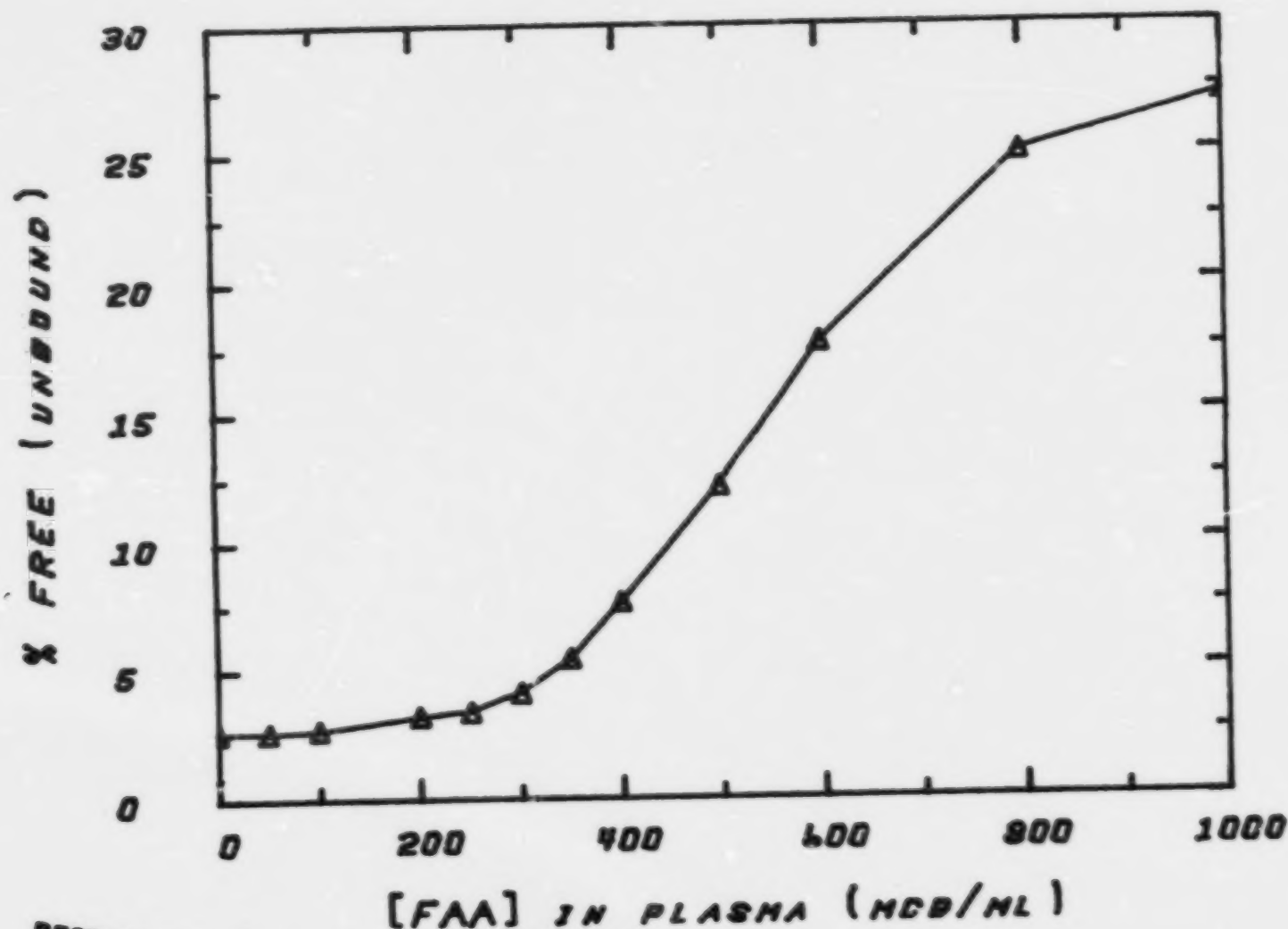
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- $\star$ 3000 mg/sq.m
- $\circ$ 1650 mg/sq.m
- $\triangle$ 1100 mg/sq.m
- $\square$ 660 mg/sq.m
- $\diamond$ 330 mg/sq.m

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PLASMA PROTEIN BINDING OF FAA

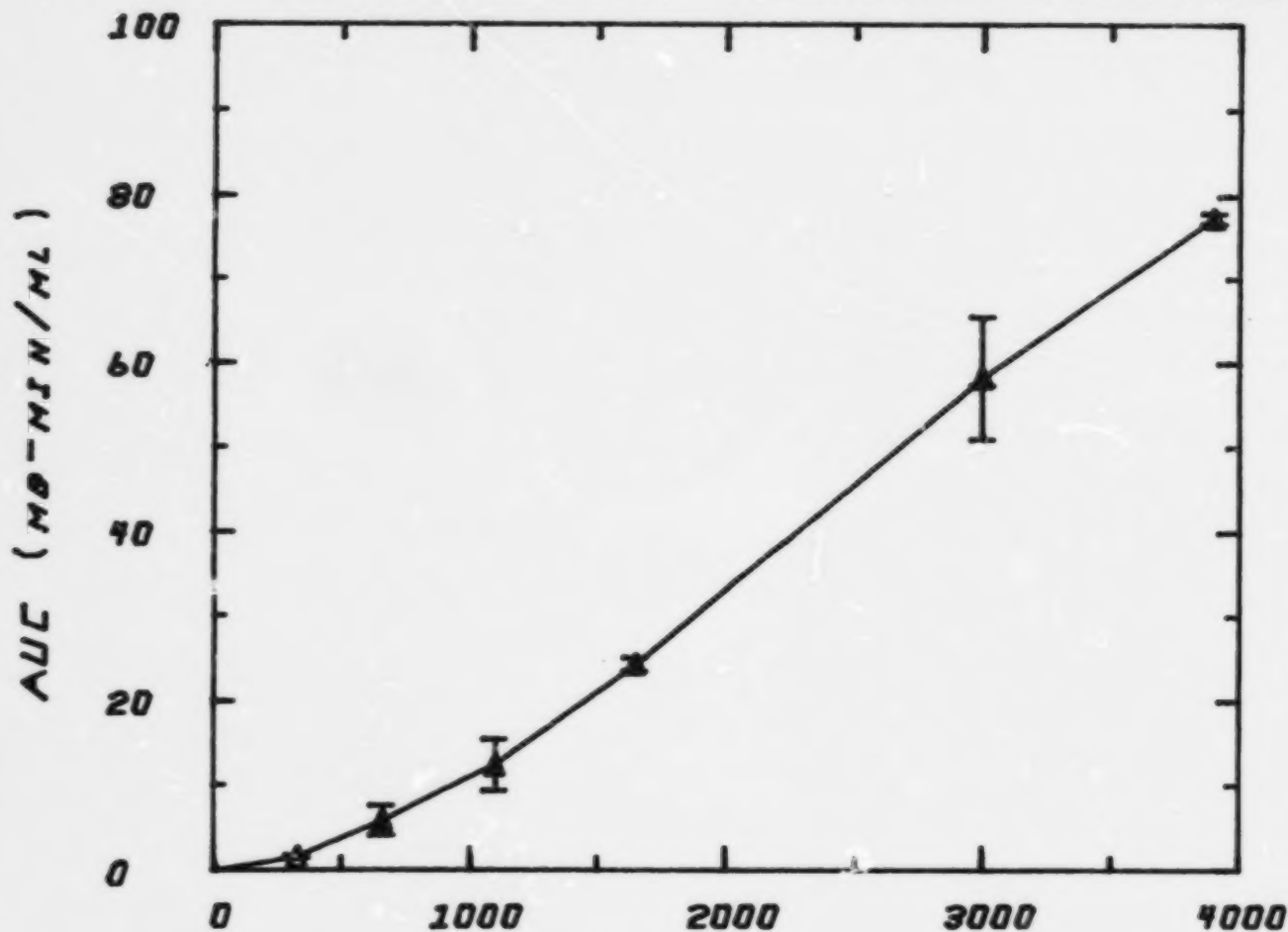


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FLAVONE ACETIC ACID DOSE DEPENDENT PHARMACOKINETICS



FAA DOSE (mg/kg)

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"EQUIVALENT" CONCENTRATIONS IN MOUSE AND HUMAN PLASMA

[FLAVONE ACETIC ACID] ($\mu\text{g/ml}$)

<u>HUMAN</u>	<u>MOUSE</u>	<u>RATIO</u>
491	213	2.3
263	88	3.0
150	46	3.3

San Antonio Phase I Trial of Flavone Acetic Acid

1. Schedule: weekly x 4; repeat every 5 weeks.
2. Prehydration: 2 liters 0.5 NS + 1 amp NaHCO_3 over 8 hours
3. Administration: 1 hour infusion in 250 cc NS (total dose ≤ 6 gm)
2 hour infusion in 500 cc NS (total dose ≥ 6 gm)
4. All treatment courses as inpatients; minimum hospitalization of 24 hours after therapy.

DOSE ESCALATION SCHEMA/PATIENT ACCRUAL

<u>Dose (mg/m²)</u>	<u>No. of Patients</u>
330	3
660	3
1100	3
2310	4
3000	4
3900	1

PATIENT CHARACTERISTICS

Number of patients

Male/Female	13/4
Age (yrs)	
Median	65
Range	38 - 76

Performance Status

0 - 1	10
2 - 3	7

Previous Treatment

None	3
Radiation only	1
Chemothe. apy only	3
Radiation + Chemotherapy	10

TUMOR TYPE

<u>Type</u>	<u>No. Patients</u>
Colorectal	6
NonSmall-Cell Lung	4
Sarcoma	2
Endometrial	1
Renal	
Breast	
Pancreas	
Small-Cell Lung	

TOXICITY

1. FAA at doses $\leq 3000 \text{ mg/m}^2$ - nausea in 6/13
2. At $>3000 \text{ mg/m}^2$:
 - 2/3 sensation of warmth, flushing during the infusion
with no change in vital signs
 - 1/3 nausea and vomiting at the end of infusion
 - 1 hypersensitivity reaction

TOXICITY

65 y/o female with metastatic endometrial carcinoma treated with FAA-3000 mg/m^2 . Fifteen minutes into the infusion, the patient complained of "runny" eyes. Subsequently developed periorbital and lip edema. Infusion stopped and IV diphenhydramine administered. Edema completely resolved at 24 hours. No change in blood pressure or respiratory status.

RESPONSE

64 y/o male with malignant fibrous histiocytoma received $330 \text{ mg/m}^2 \times 4$ for 2 courses and had a 30% decrease in lung metastases and the primary tumor. Therapy discontinued after 2 courses secondary to patient request and declining performance status.

Activity of Flavone Acetic Acid in a Human Tumor

Cloning System

<u>Concentration (μm)</u>	<u># Response/# Evaluable</u>	<u>Tumor Type</u>
0.1	0/12	----
1.0	0/12	----
10.0	0/12	----
200.0	1/12	Ovarian
1000.0	4/12	Ovarian, renal, breast, brain

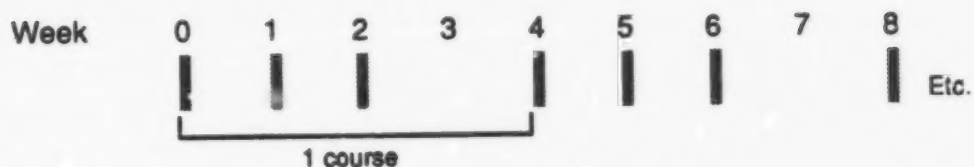
SUMMARY - SAN ANTONIO PHASE I TRIAL OF FLAVONE ACETIC ACID

- I. Schedule - weekly x 4 q 5 weeks
- II. Prehydration/monitoring - 2 liters 0.5 NS + 1 amp NaHCO_3 over 8 hrs; inpatient administration and 24 hr posttreatment monitoring
- III. Present dose: 3900 mg/r^{-2}
- IV. Toxicity - Nausea, vomiting, sporadic at $\leq 3000 \text{ mg/m}^2$; at 3000 mg/m^2 nausea, vomiting and/or flushing in 3/3 pts; hypersensitivity reaction in one patient at 3000 mg/m^2
- V. Tumor response: MR (30% regression) at 330 mg/m^2 of fibrous histiocytoma lasting 10 wks
- VI. Plan: Continue dose escalation

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STUDY DESIGN

Schedule: I.V. bolus weekly x 3



Patients: 1 to 21 years of age
Refractory cancer (solid tumors or leukemia)
Recovered from toxic effects of prior therapy
Adequate hepatic and renal function

Dose: Starting dose - 35 mg/m²
Escalate by 30% - 50, 65, 85, 110, 145 mg/m²
Minimum of 3 patients at each level (2 without marrow disease)

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PATIENT CHARACTERISTICS

No. of evaluable patients	30
No. of courses	37
Age (yr)	
Median	13
Range	3-20
Mean ECOG ^a performance score	1.6
No. of patients with prior therapy	
Chemotherapy alone	8
Radiotherapy alone	1
Chemotherapy + radiotherapy	21
Diagnosis	
Brain tumors	11
Acute lymphoblastic leukemia	7
Osteosarcoma	3
Acute nonlymphocytic leukemia	2
Lymphoma	1
Ewings sarcoma	1
Neuroblastoma	1
Teratoma	1
Adenocarcinoma	1
Wilms' tumor	1
Synovial cell sarcoma	1

^a ECOG, Eastern Cooperative Oncology Group

RESULTS OF PHASE I TRIAL

Weekly dose (mg/m ²)	No. of patients/ no. of courses	No. with dose-limiting toxicity	Nature of toxicity
35	3/3	0	
50	4/5	1	Thrombocytopenia
65	4/6	0	
85	7/7	0	
110	6/6	2	Pancytopenia (2), mucositis, renal and hepatic(?) dysfunction
145	6/6	3	Pancytopenia (2), rash (2), mucositis

DOSE-LIMITING TOXICITY IN A PATIENT AT THE 110 MG/M² DOSE LEVEL

Patient: 17 yo male with adenocarcinoma of the colon who had recurrent abdominal disease and ascites.

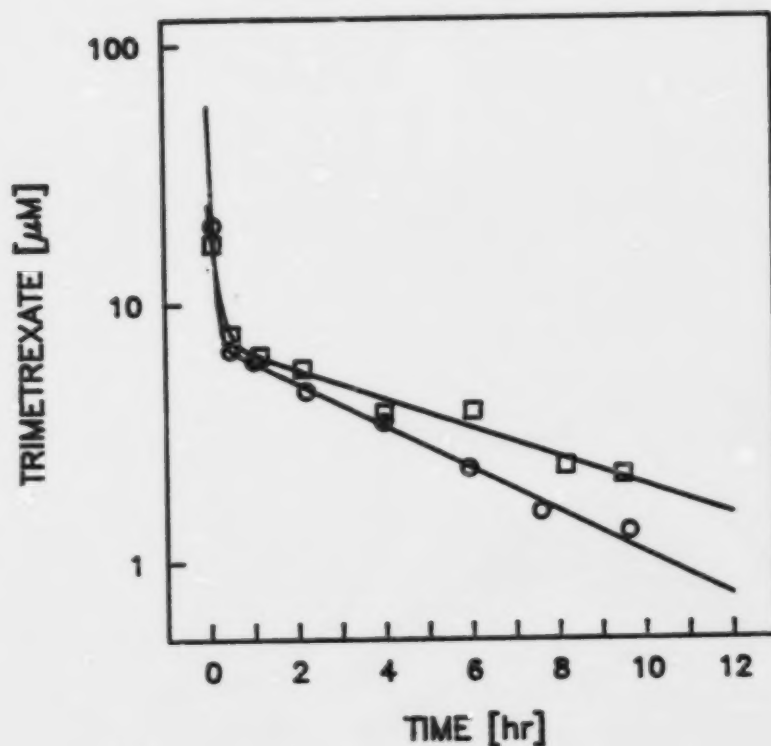
Toxicity: Grade 4 neutropenia & thrombocytopenia (5 d post 1st dose)
Grade 3 mucositis
Grade 3 renal (creatinine 1.1 to 2.9 mg/dl, 10 d post 1st dose)
Grade 3 (?) hepatic (bili 0.5 to 5.8 mg/dl, 10 d post 1st dose)

PK Parameters: C_{total} = 10.8 ml/min/m² (mean = 40.1)
C_{renal} = 4.6 ml/min/m² (mean = 4.3)
t_{1/2} = 20 hr (mean 7.6)
C₂₄ = 5.4 μM, C₄₈ = 3.1 μM

Drug Interaction: Patient receiving continuous infusion morphine (30 mg/hr)

Effect of morphine on the clearance of trimetrexate in 2 Rhesus monkeys. Points represent the mean of 2 animals. Each received trimetrexate alone (o) and trimetrexate with a short infusion of morphine (). $T^{1/2}$ was twice as long and clearance was 30 to 40% less when the animals were given morphine with trimetrexate.

EFFECT OF MS ON TRIMETREXATE PLASMA LEVELS



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PHARMACOKINETIC PARAMETERS OF TRIMETREXATE IN CHILDREN.

Dose	n	AUC ($\mu\text{M}\cdot\text{h}$)	C _I total ($\text{ml}/\text{min}/\text{m}^2$)	C _I renal ($\text{ml}/\text{min}/\text{m}^2$)	Vd _{ss} (L/m^2)	T _{1/2} (h)	C ₂₄ ^a (μM)	Urinary Excretion (%)
35	5	64.2 $\pm$ 63.7 ^b	47.5 $\pm$ 37.7	3.2 $\pm$ 3.5	11.8 $\pm$ 9.7	5.3 $\pm$ 2.5	0.40 $\pm$ 0.68	8.6 $\pm$ 7.0
50	5	76.0 $\pm$ 33.1	37.3 $\pm$ 23.0	8.9 $\pm$ 2.5	12.6 $\pm$ 7.5	6.7 $\pm$ 2.8	0.41 $\pm$ 0.27	14.7 $\pm$ 7.7
65	4	116 $\pm$ 44	28.8 $\pm$ 12.4	3.1 $\pm$ 3.7	8.4 $\pm$ 2.7	7.6 $\pm$ 2.0	0.69 $\pm$ 0.75	9.2 $\pm$ 9.6
85	4	176 $\pm$ 106	32.9 $\pm$ 26.3	2.7 $\pm$ 0.9	8.9 $\pm$ 2.2	7.7 $\pm$ 2.2	0.97 $\pm$ 1.0	15.6 $\pm$ 1.3
110	4	309 $\pm$ 140	19.1 $\pm$ 9.1	4.3 $\pm$ 3.0	15.0 $\pm$ 3.9	12.0 $\pm$ 5.5	3.1 $\pm$ 2.0	20 $\pm$ 13
145	3	225 $\pm$ 174	85.7 $\pm$ 113	4.5 $\pm$ 5.1	17.0 $\pm$ 8.7	7.1 $\pm$ 3.4	2.1 $\pm$ 1.5	8.0 $\pm$ 3.6
Mean	25		40.1	4.3 ^c	12.1	7.6		12.6 ^c
SD			43.3	3.6	6.6	3.6		8.6
Range			9.2-215	0.2-11.4	3.4-26.8	2.1-20		0.6-31

^a C₂₄, Concentration of trimetrexate at 24 h.

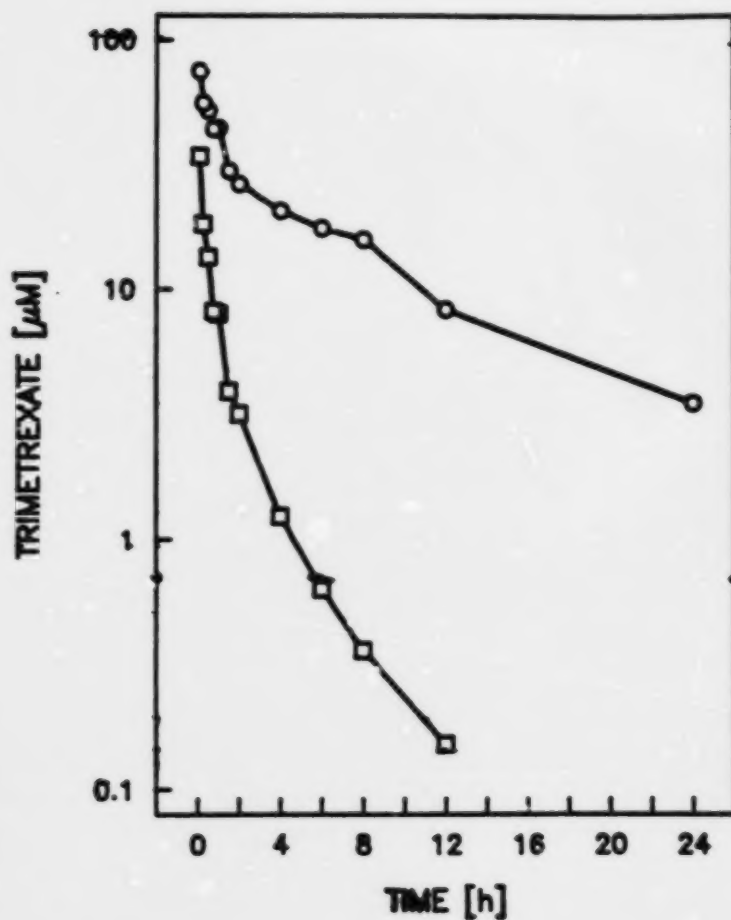
^b Mean $\pm$ SD.

^c n = 22, there were 3 incomplete urine collections, 2 at the 50 mg/m² dose level and 1 at the 85 mg/m² dose level

Trimetrexate
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Plasma disappearance curves from 2 patients treated at the 145 mg/m² dose level
illustrating interpatient variability observed



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CONCLUSIONS

- The MTD of trimetrexate in children on a weekly i.v. bolus schedule is 110 mg/m²
- The dose-limiting toxicities are myelosuppression, rash and mucositis
- The pharmacokinetic behavior of trimetrexate is highly variable with a wide range in drug clearance and half-life
- There is a possible interaction between trimetrexate and morphine which may enhance the toxicity of trimetrexate when these 2 drugs are co-administered

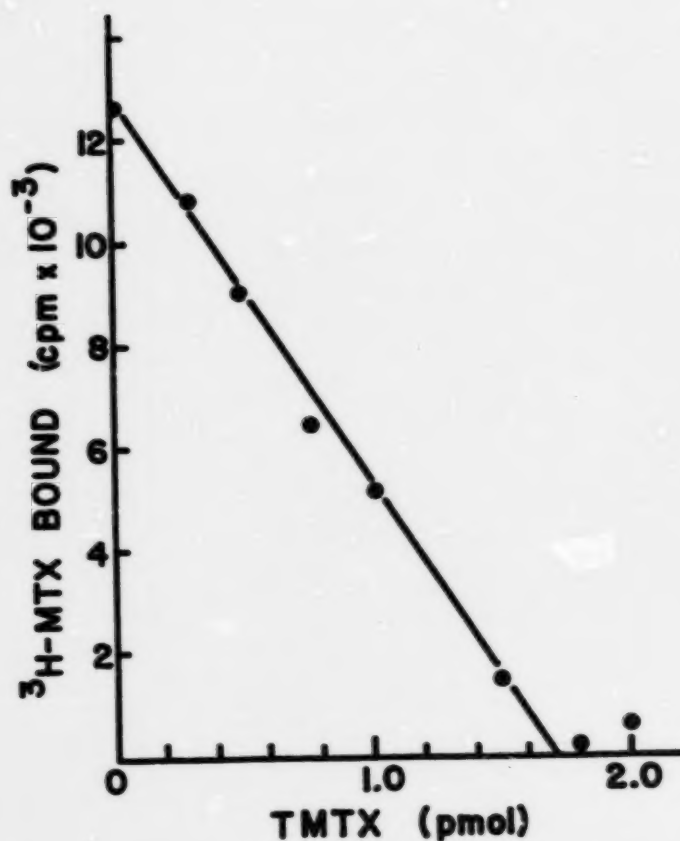
TREATMENT PLAN

- I. 30 minute infusion daily X 5 days, repeated every 21 days.
- II. Dose: 6.4 mg/m²
7.7 mg/m²
9.2 mg/m²
- III. Pharmacokinetics studied 1st and 5th dose.

ASSAYS

- I. DHFR, ³H-MTX and TMTX in a "sequential" saturation mode.
Sensitivity .1 - .2 pmol

Confirmed by
- II. HPLC and spectral detection (J.J. McCormack, J. Stewart and B. Tong)



"Standard curve" for TMX assay. Dihydrofolate reductase is incubated with TMTX (or sample) for 30'-60' and then ³H-MTX is added for additional 10'. Bound and free ³H-MTX are separated using charcoal coated with Dextran as noted elsewhere.
(Kamen et al *Anal Biochem* 70:54, 1976)

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PATIENTS

N = 16 (Female 9; Male 7)

Age 10 (range 4-15)

Diseases

Leukemia: 12

ALL 9

T-ALL 1

M5 1

M7 1

Solid Tumor 4

Lymphoma 1

Glioma 1

Glioblastoma 1

Renal cell carcinoma 1

All patients, except for teenager with renal cell carcinoma, had significant prior multi-agent chemotherapy.

TOXICITY

<u>Dose</u>	<u>Leukemia patients</u>	<u>Courses</u>	<u>Toxicity</u>
6.4	4	4	Mucositis 1 (mild) n/v 1 (mild)
7.7	4	4	Skin rash 1 (moderate) mucositis 1 (severe)
9.2	4	5	Mucositis 3 (moderate to severe)
<u>Solid Tumor</u>			
6.4	2	11 (1 + 10)	--
7.7	2	6 (1 + 5)	Mild n/v

No major hematologic toxicity (platelet <50,000, WBC < 1500) seen in patients with a solid tumor.

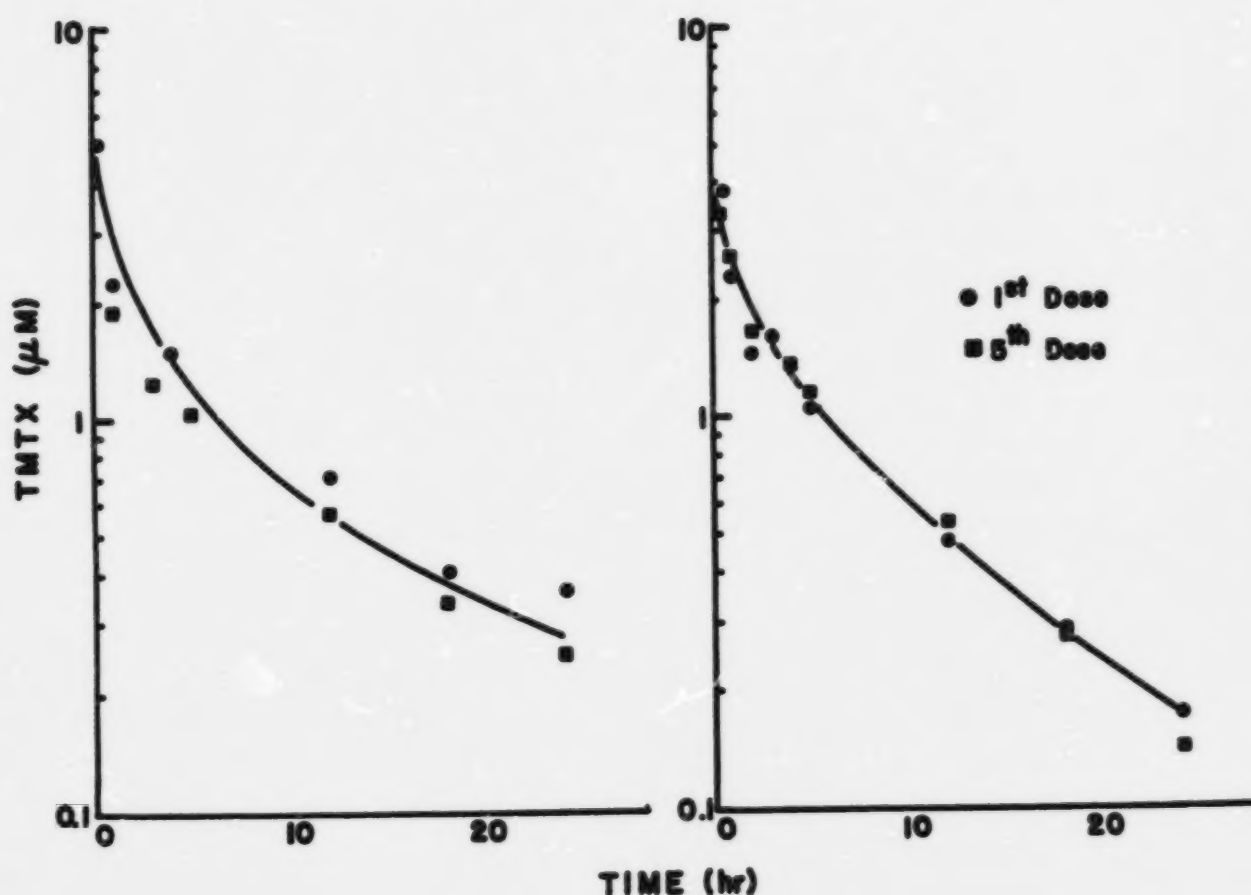
RESPONSE

Leukemia - 12 patients

"oncolytic" - 7
PR - 1

Solid tumor - 4 patients

glioma PR (10 courses, 1 yr) +
renal cell SD (5 courses, 4 months)



Typical plasma clearance of TMTX following a 30' infusion. Two patients (one each at 6.4 mg/m² and 7.7 mg/m²) were studied during the 1st and 5th dose. There is little inpatient variability. The T_{1/2} for 16 infusions is 320 +/- 28 minutes (95% CL).

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CONCLUSIONS

- I. Acute toxicity minimal.
- II. DLT in patients with leukemia, all heavily pretreated is mucositis.
- III. DLT for solid tumor patients not yet reached.
- IV. Little inter/intrapatient variability in pharmacokinetic studies.
- V. Recommended starting dose for leukemia patients approximately 7.7 mg/m^2 .

MERBARONE

PATIENT CHARACTERISTICS

NUMBER PATIENTS	12
MEDIAN AGE	48 (RANGE 44-65)

INITIAL PERFORMANCE STATUS (PS)

<u>PS</u>	<u>NUMBER OF PATIENTS</u>
0	4
1	2
2	0
3	6
4	0

PRIOR THERAPY

CHEMOTHERAPY	12
RADIATION THERAPY	4

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Merbarone
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MERBARONE			
DRUG ADMINISTERED			
DOSE LEVEL (MG/M2) CIV X 5 DAYS	DOSE LEVEL (MG/M2) 2-HOUR CIV X 5 DAYS	NUMBER OF PATIENTS	NUMBER COURSES
96	-	3	6
144	-	3	4
192	-	4	6
-	150	2	2

MERBARONE	
DISEASE CATEGORY	
HEAD AND NECK CARCINOMA	3
KIDNEY CARCINOMA	3
ADENOCARCINOMA COLON	3
BLADDER CARCINOMA	1
LUNG CARCINOMA	1
OVARIAN CARCINOMA	1

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MERBARONE

NONHEMATOLOGIC TOXICITY

DOSE LEVEL (MG/M2) CIV X 5 DAYS	DOSE LEVEL (MG/M2) 2-HOUR CIV X 5 DAYS	NUMBER COURSES	<u>EPISODES TOXICITY (MEDIAN GRADE)</u>	
			<u>GASTROINTESTINAL</u>	<u>PERITONITIS</u>
96	-	6	3 (1)	2 (2)
144	-	4	2 (1)	2 (2)
192	-	6	5 (2)	3 (2)
	150	2	-	1 (1)

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MERBARONE

RATIONALE FOR NEW DOSE SCHEDULE

THE OBSERVATION OF GRADE 2 PHLEBITIS IN THE CONTINUOUS INFUSION SCHEDULE, DESPITE INCREASED FLUIDS, PRECLUDED FURTHER ADMINISTRATION OF THIS AGENT THROUGH A PERIPHERAL LINE. THE PROTOCOL WAS AMENDED TO REQUIRE SUBSEQUENT ADMINISTRATION THROUGH A LARGE CENTRAL LINE.

ADDITIONALLY, A SECOND SCHEDULE FOR MERBARONE ADMINISTRATION WAS INITIATED USING A SHORT INFUSION OVER TWO HOURS. TWO PATIENTS HAVE TOLERATED THIS SCHEDULE WITH ONLY MILD ERYTHEMA ALONG THE VEIN ON DAY ONE OF ONE PATIENT.

SUMMARY

1. THE OBSERVATION OF PHLEBITIS IN THE CONTINUOUS INFUSION SCHEDULE PRECLUDED FURTHER ADMINISTRATION OF THIS AGENT THROUGH A PERIPHERAL LINE REQUIRING THAT SUBSEQUENT ADMINISTRATIONS BE ACCOMPLISHED THROUGH A CENTRAL VENOUS CATHETER.
 2. A SECOND SCHEDULE FOR MERBARONE ADMINISTRATION WAS INITIATED USING A SHORT INFUSION OVER TWO HOURS. TWO PATIENTS HAVE RECEIVED THIS SCHEDULE WITH MINIMAL TOXICITY.
 3. OUR PLANS INCLUDE: 1) CONTINUED ESCALATION OF THE CONTINUOUS INFUSION SCHEDULE THROUGH A CENTRAL VENOUS CATHETER TO MONITOR FOR OTHER TOXICITIES. 2) MONITOR 2-HOUR INFUSION SCHEDULE FOR PHLEBITIS AS WELL AS OTHER TOXICITIES.
-

MERBARONE

Pharmacology Report

THE OHIO STATE UNIVERSITY

A. INTRODUCTION

Clinical pharmacokinetic studies have been conducted in conjunction with the phase I evaluation of merbarone (NSC 336628, MB). The present report summarizes the plasma pharmacokinetics in 9 patients during and following the five day 24 hour continuous infusion of the drug, the plasma pharmacokinetics in one patient treated with a 2 hour infusion daily for 5 days, and further studies to characterize the human metabolites of MB.

Phlebitis was observed in patients during the 24 hour continuous infusion daily x 5 schedule at this institution and at the Memorial Sloan Kettering Cancer Center. Accordingly, the clinical protocol for this schedule was amended requiring that the drug be administered via a Hickman catheter for new patients entered on this schedule. Additionally, a 2 hour infusion daily x 5 schedule was initiated for evaluation.

B. METHODS

1. Analytical Method

The reversed-phase HPLC method developed for the quantitation of plasma and urine levels of MB was described in detail at the Phase I Working Group meeting of July, 1986.

2. Clinical Pharmacokinetics

The blood sampling protocol and the methods for analyzing the plasma pharmacokinetics of MB in patients on the 24 hour continuous infusion daily x 5 schedule were previously described. It was not until the second dose level (144 mg/m²/day x 5), that sampling and timing techniques were established which permitted appropriate fitting of the data.

For the 2 hour infusion daily x 5 schedule, blood sampling is carried out prior to each dose and on day 5 at 15 minute intervals during the course of the infusion and at frequent intervals to 72 hours following the end of the infusion. The plasma concentrations of merbarone as a function of time were NONLIN fitted using weights of $1/y^2_{obs}$ to a two compartment open model with a continuous zero-order infusion input.

3. Metabolite Characterization

UV spectra during chromatography were obtained with a Hewlett-Packard model 1040M diode array detector. Spectra were scanned from 210 nm to 400 nm during each 160-200 msec of the chromatograms. Chromatography was conducted with the analytical mobile phase and Nova-Pak C₁₈ columns.

Chemical ionization and electron impact mass spectra were obtained using a direct insertion probe with a Kratos model MS-25RFA spectrometer.

MB and its metabolites were isolated from patient urine employing the solid phase extraction technique. Studies undertaken to separate the compounds by preparative TLC have not been successful, however, significant clean-up was achieved. Mass spectra of the isolated mixture were determined in order to obtain additional evidence for structures of the metabolites tentatively characterized from LC retention times and UV spectra.

C. RESULTS AND DISCUSSION

1. Clinical Pharmacokinetic Studies

24 Hour Continuous Infusion Daily x 5

The plasma pharmacokinetics are summarized for 9 patients treated with 96, 144, and 192 mg/m²/day x 5 by 24 hour continuous infusion of merbarone.

The plasma concentration-time profile for a patient at the upper dose level is shown in Fig. 1. The curve drawn through the experimentally determined plasma concentrations is the computer fit to the data. Following the end of the infusion, the plasma levels exhibited biphasic decline.

The individual pharmacokinetic parameters at the second dose level are listed in Tables 1 - 3. Cl_T for the patients ranged from 8.01 to 21.99 ml/min/m². Following the end of the infusion, the initial half-life range was 0.2 to 3.7 hr and the terminal half-life range was 9.2 to 14.6 hr.

The central compartment volume of distribution, V₁, was small ranging from 1.9% to 18.9% of body weight (Table 2). The steady state volume of distribution, Vd_{ss}, was also relatively small ranging from 15.2% to 26.8% of body weight. Even the total body volume of distribution, Vd_B, was relatively small corresponding to 22.4% to 62.7% of body weight. Thus, it appears that merbarone is not extensively bound to deep tissue sites.

The total body clearance for 9 courses of merbarone ranged from 8.0 to 22.0 ml/min/m² (Table 3). At dose level 1, each patient was treated with two courses of the drug and for a given patient, the total body clearance for the two courses was similar (Table 3). The observation that both the total body clearance and the volumes of distribution were small suggests that merbarone is highly bound to plasma proteins.

The urinary excretion of merbarone was determined for 7 days after the start of the infusion for 7 of 13 courses administered (previously reported). The mean urinary excretion of the parent drug was 1.9% of the dose with a range of 0.3% to 4.3%. Accordingly, renal clearance of the unchanged drug is a minor component of the total body clearance.

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2 Hour Infusion Daily x 5

The plasma pharmacokinetics of MB is reported for a single patient treated with 150 mg/m²/day x 5 administered as a 2 hour infusion. The plasma concentration-time profile showed biphasic decline following the end of the infusion (Fig. 2).

The parameters determined from the computer fit of the plasma concentration-time data for this study were generally similar to the mean parameters determined at each dose level for the 144 and 192 mg/m² 24 hour CIV studies (Table 4).

For the limited number of patients which have been studied at present, the variability of the pharmacokinetic parameters between patients exceeds the range of the doses.

2. Metabolite Characterization

Liquid chromatographic and UV spectral evidence was presented in July for the presence of two major metabolites and at least one minor metabolite in the urine of patients treated with merbarone. The major metabolites were tentatively identified to be 4'-hydroxymerbarone and 2-oxo-2-desthiomerbarone (Fig. 3). During recent months, efforts to isolate and separate the major urinary metabolites have not been successful, however, mass spectral evidence has been obtained to support the proposed structures. In addition, we have determined that there is also present a third major urinary metabolite previously masked by endogenous constituents.

Our previous report demonstrated that chromatograms of patient urine injected directly on column employing the analytical column and mobile phase, showed the presence of two major metabolites, one which elutes shortly after the solvent front, where urinary constituents are present, corresponding to the retention time of 4'-hydroxymerbarone, and another which precedes the elution of the parent drug exhibiting a retention time identical to that of 2-oxo-2-desthiomerbarone (Fig. 4). The UV absorption maximum of the latter metabolite occurs at approximately 278 nm and a chromatogram determined at this wavelength demonstrates that it is a prominent metabolite (Fig. 5). In addition, the chromatograms show peaks which are likely due to three minor metabolites, one preceding 2-oxo-2-desthiomerbarone and two following merbarone (Fig. 4-6).

The chromatogram obtained at 279 nm suggested that a metabolite was obscured by the presence of the polar constituents of urine (Fig. 5). Therefore, a solid phase extraction system was developed for sample isolation and clean-up. The mixture obtained showed the chromatographic peaks, using 306 nm detection and a new Nova-Pak C₁₈ column, at 1.4 min, 2.0 min (4'-hydroxymerbarone), 5.1 min (2-oxo-2-desthiomerbarone), and 7.1 min (merbarone) (Fig. 7). The minor metabolite preceding the 2-oxo-2-desthiomerbarone was present, however those following merbarone were not (Fig. 8).

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In this sample, the predominant metabolite was 2-oxo-2-desthiomerbarone (Fig. 9) and the unknown new metabolite eluting at 1.4 min was present in an amount approximately equal to that of 4'-hydroxymerbarone (Fig. 10). The unknown metabolite was found to have a UV spectrum characteristic of the 2-oxo compounds (Fig. 11) and dissimilar from that of the 2-thio compounds such as merbarone (Fig. 12) and 4'-hydroxymerbarone (Fig. 13). An overlay of the spectrum of the unknown metabolite and that of 2-oxo-2-desthiomerbarone (Fig. 14) demonstrates the similarity of the spectra. Thus, the new major metabolite is considered to be a 2-oxo-2-desthiomerbarone derivative.

Table 5 shows the parent ions and the M+1 ions which were present in electron impact and chemical ionization mass spectra respectively. These spectra showed the presence of ions corresponding to the tentatively identified metabolites, 4'-hydroxymerbarone and 2-oxo-2-desthiomerbarone providing additional evidence that these are major metabolites.

D. PHARMACOKINETIC AND METABOLIC SUMMARY

1. The mean total body plasma clearance, Cl_T , of merbarone during the 5 day continuous infusion of 96 to 192 mg/m²/day was 12.5 ml/min/m² (c.v. 33%) for 9 patients.

Cl_T for one patient treated with 150 mg/m² as a two hour infusion daily x 5 was 8.3 ml/min/m².

2. The decline of the plasma levels following the end of the infusion was biphasic. For 6 patients on the CIV schedule, the harmonic mean half-lives were: $t_{1/2\alpha} = 0.75$ hr and $t_{1/2\beta} = 11.26$ hr.
3. Merbarone is not highly bound to body tissues. The mean V_{dss} was 7.87 L/m² (21 % of body weight).
4. Elimination is primarily non-renal (approximately 2% of the dose, previously reported).
5. Chromatograms of patient urine show the presence of two major metabolites exhibiting LC retention times and UV spectra similar to 4'-hydroxymerbarone and 2-oxo-2-desthiomerbarone.

Chemical ionization and electron impact mass spectra afford additional evidence for the presence of these metabolites.

Following isolation from urine, liquid chromatograms show the presence of a third major urinary metabolite with a UV spectrum suggesting that it is a 2-oxo-2-desthiomerbarone derivative.

Thus, three major and at least two minor metabolites are present in patient urine.

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TABLE 1

MERBARONE HALF-LIVES, MEAN RESIDENCE TIMES AND PERCENT OF THE AREA CORRESPONDING TO THE PHASES UNDER THE PLASMA CONCENTRATION-TIME CURVE FOLLOWING 24 HOUR CONTINUOUS IV INFUSION DAILY X 5

Patient	Dose (mg/m ² /d)	t _{1/2α} (hr)	t _{1/2β} (hr)	I.V. BOLUS MRT (hr)	α-Phase AUC,%	β-Phase AUC,%
4	144	3.71	14.55	10.23	68.81	31.19
5	144	0.95	10.14	7.31	55.14	44.86
6	144	0.77	9.16	8.95	35.24	64.76
7	192	4.04	12.51	13.01	41.20	58.80
8	192	2.43	12.19	7.50	71.66	28.34
9	192	0.21	10.62	11.36	26.39	73.61

TABLE 2

MERBARONE VOLUME PHARMACOKINETIC PARAMETERS FOLLOWING 24 HOUR CONTINUOUS IV INFUSION DAILY X 5

Patient	Dose (mg/m ² /d)	V ₁ (L/m ²)	V ₂ (L/m ²)	Vd _{ss} (L/m ²)	Vd _β (L/m ²)
4	144	5.92	2.76	8.69	17.82
5	144	1.99	4.34	6.33	12.65
6	144	1.71	3.91	5.62	8.30
7	192	7.01	2.42	9.43	13.08
8	192	5.99	3.91	9.90	23.21
9	192	0.71	6.53	7.23	9.76

TABLE 3

MERBARONE STEADY STATE PHARMACOKINETICS

Patient	Course	Dose (mg/m ² /d)	Cp _{ss} (μg/ml)	Cl _T (ml/min/m ²)
1	1	96	6.28 ^a	11.01
	2	96	5.30 ^a	12.05
2	1	96	8.31 ^a	8.01
	2	96	7.33 ^a	9.01
3	1	96	6.60 ^a	10.19
	2	96	7.11 ^a	9.19
4	1	144	7.03 ^b	14.16
5	1	144	7.03 ^b	14.42
6	1	144	9.07 ^b	10.46
7	1	192	10.77 ^b	12.08
8	1	192	5.84 ^b	21.99
9	1	192	12.65 ^b	10.61
	2	192	10.18 ^b	13.33

^aGeometric mean Cp_{ss} calculated from days 2-5 steady state plasma levels.

^bCalculated from NONLIN fit of steady state and post-infusion plasma levels.

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TABLE 4

MERBARONE MEAN PHARMACOKINETIC PARAMETERS^a

Parameter	Units	24 Hour Continuous Infusion		2 Hour Infusion	
		(mg/m ² /d x 5)		(mg/m ² /d x 5)	
		96 (n=3)	144 (n=3)	192 (n=3)	150 (n=1) ^b
Cl _T	ml/min/m ²	9.74	13.01	14.89	8.30
t _{1/2α}	hr	-	1.14	0.56	3.15
t _{1/2β}	hr	-	10.85	11.71	13.63
MRT (IV bolus)	hr	-	8.66	10.06	15.23
V ₁	L/m ²	-	3.21	4.57	4.96
V ₂	L/m ²	-	3.67	4.29	2.63
Vd _{ss}	L/m ²	-	6.88	8.85	7.59
Vd _β	L/m ²	-	12.92	15.35	9.80

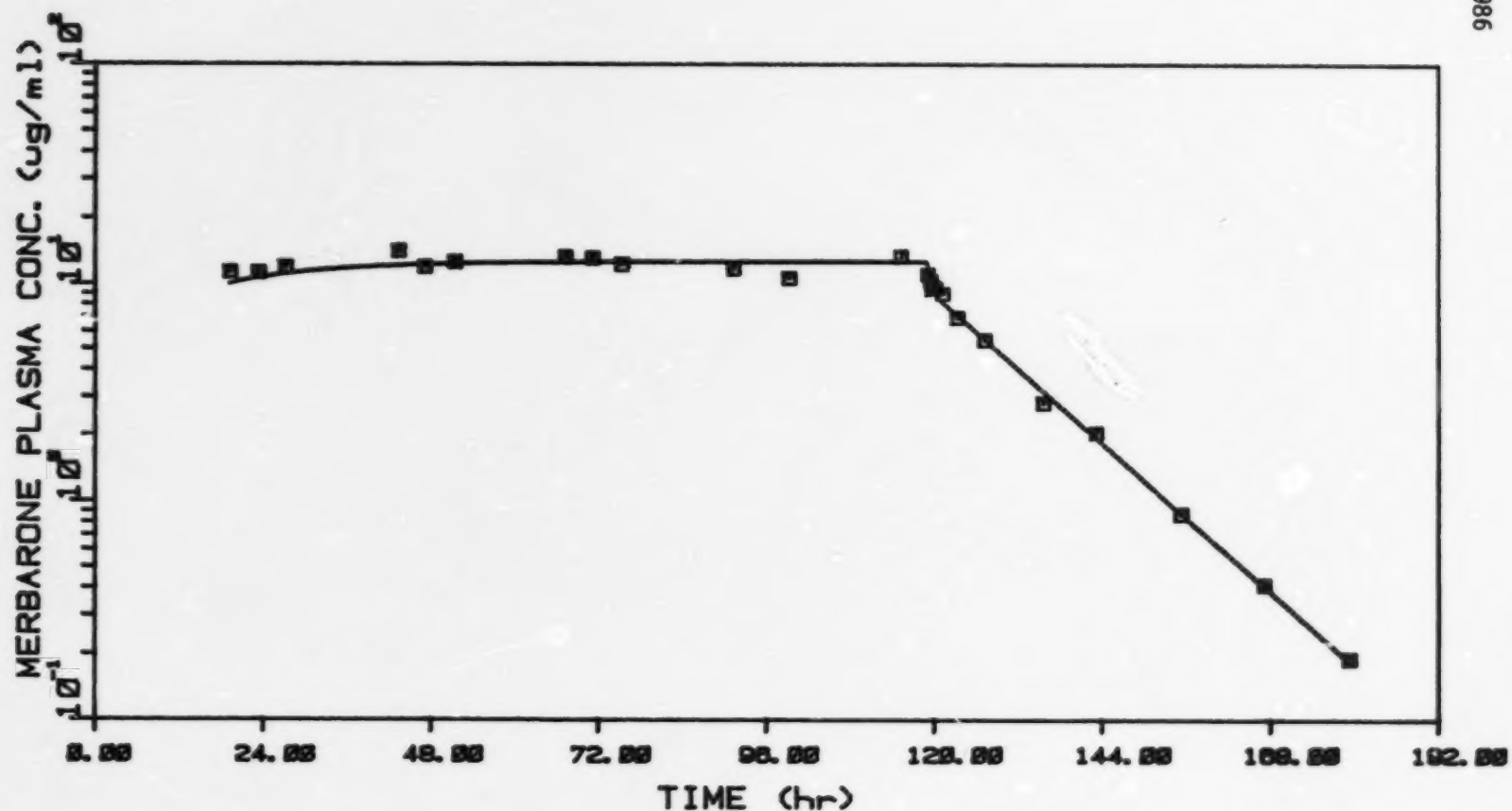
^aFirst course.

^bObserved C_{pmax} = 25.22 µg/ml. Projected C_{pss} by CIV = 12.54 µg/ml.

TABLE 5

CHEMICAL IONIZATION AND ELECTRON IMPACT MASS SPECTRA OF A MIXTURE ISOLATED FROM HUMAN URINE

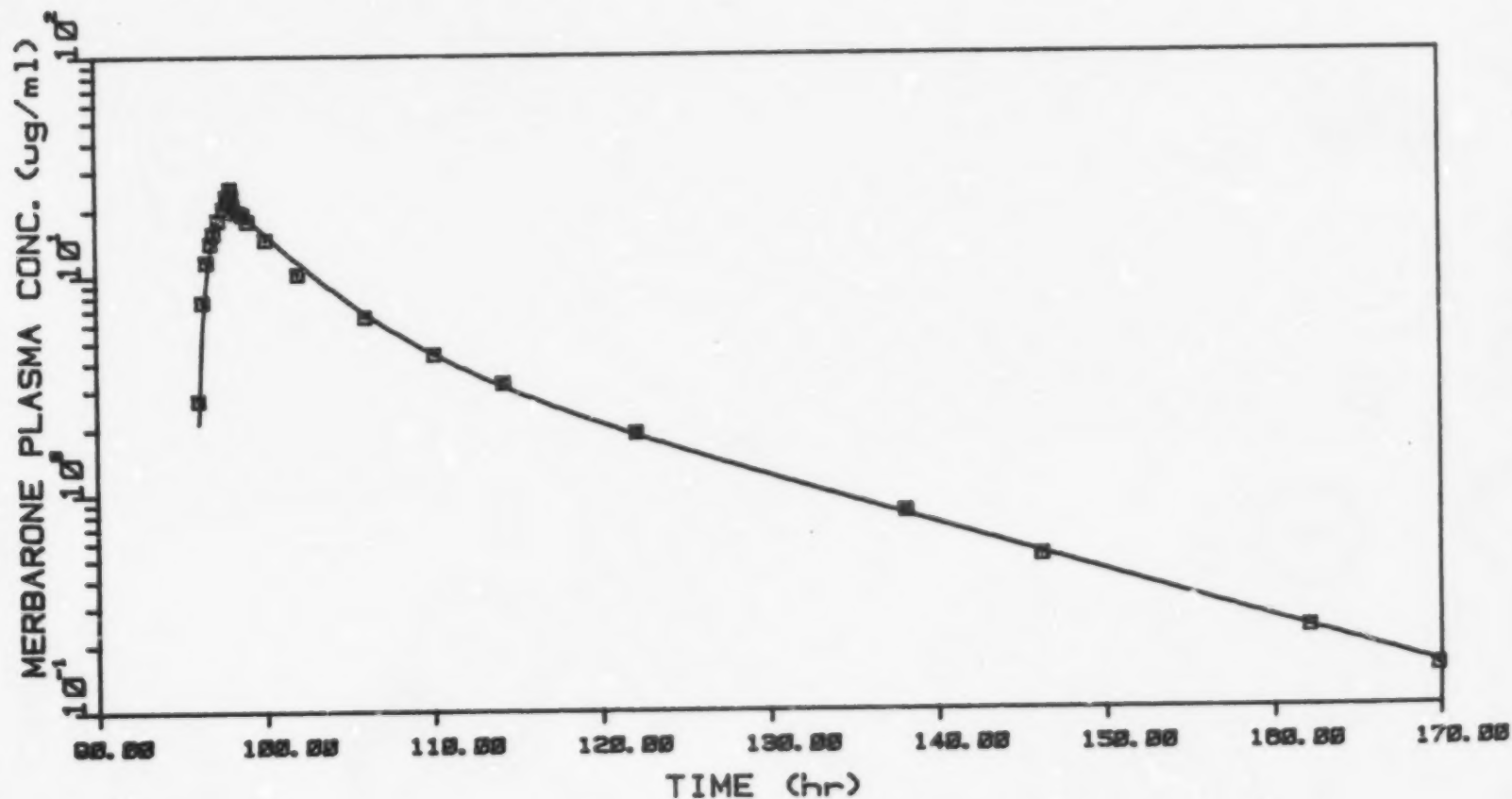
Metabolite	E.I.	C.I.
	M ⁺	M+1
2-Oxo-2-desthiomerbarone	247	248
Merbarone	263	264
4'-Hydroxymerbarone	279	280
Unknown	293	294



PATIENT J. S.
DOSE = 192 MG/M²/DAY × 5 MERBARONE (CIV)
C_{Pss} = 12.65 ug/ml

Fig. 1

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PATIENT V.P.

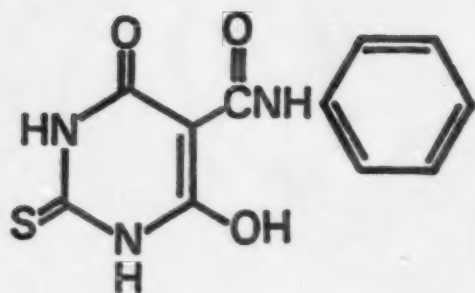
DOSE = 150 MG/METER SQ./DAY × 5 (2 HR INF.)

PROJECTED CP_{ss} = 12.54 ug/ml

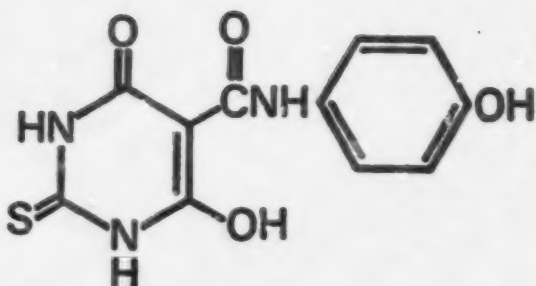
Fig. 2

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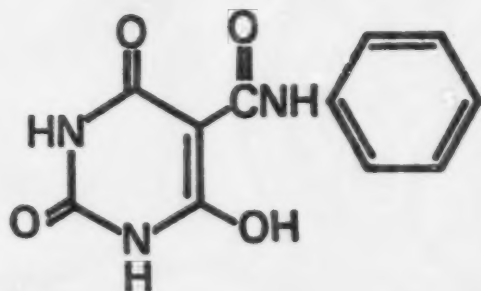
STRUCTURES OF MERBARONE AND ITS METABOLITES IN MAN



MERBARONE



4'-HYDROXYMERBARONE



2-OXO-2-DESTHIOMERBARONE

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Fig. 3

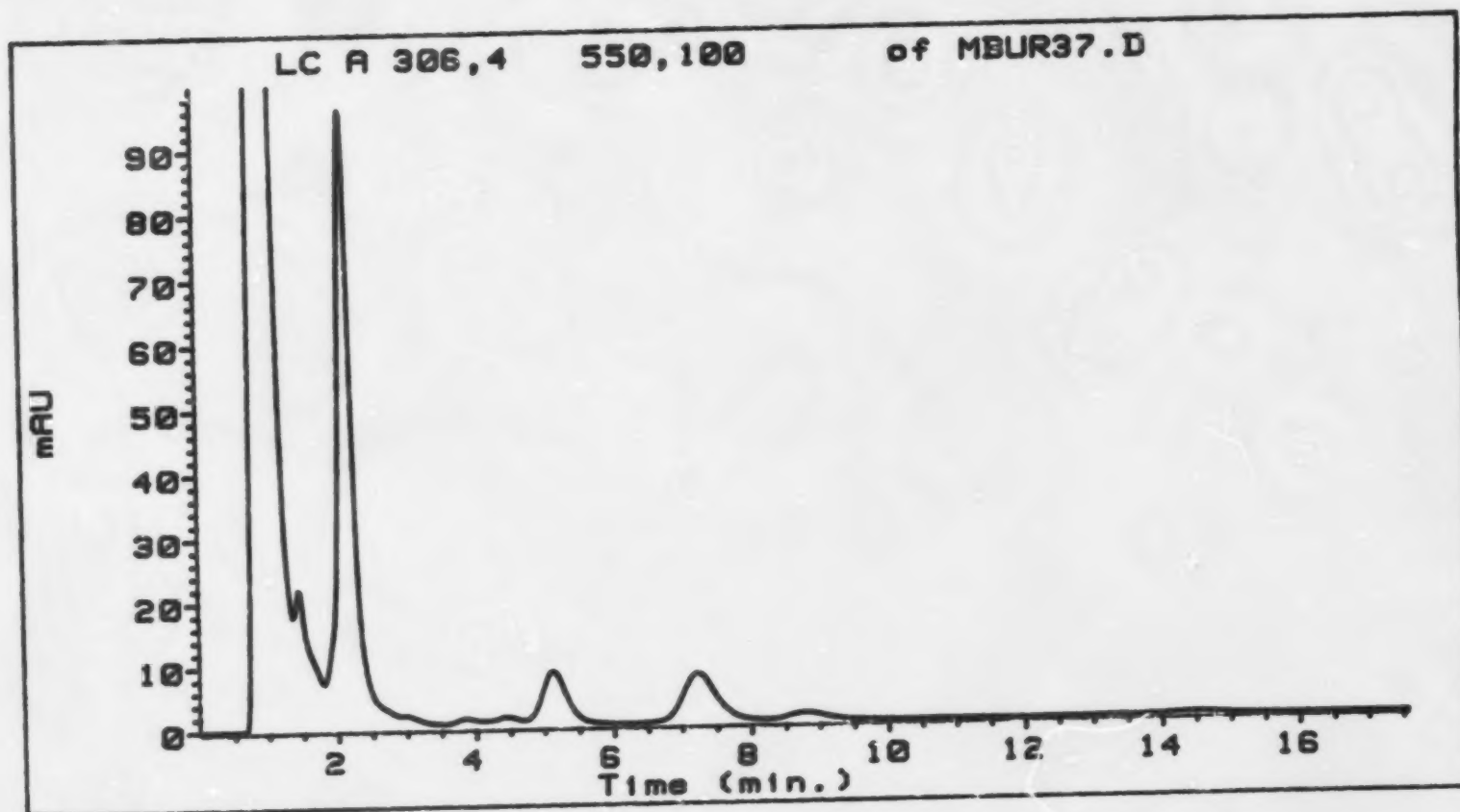


Fig. 4

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Merbarone
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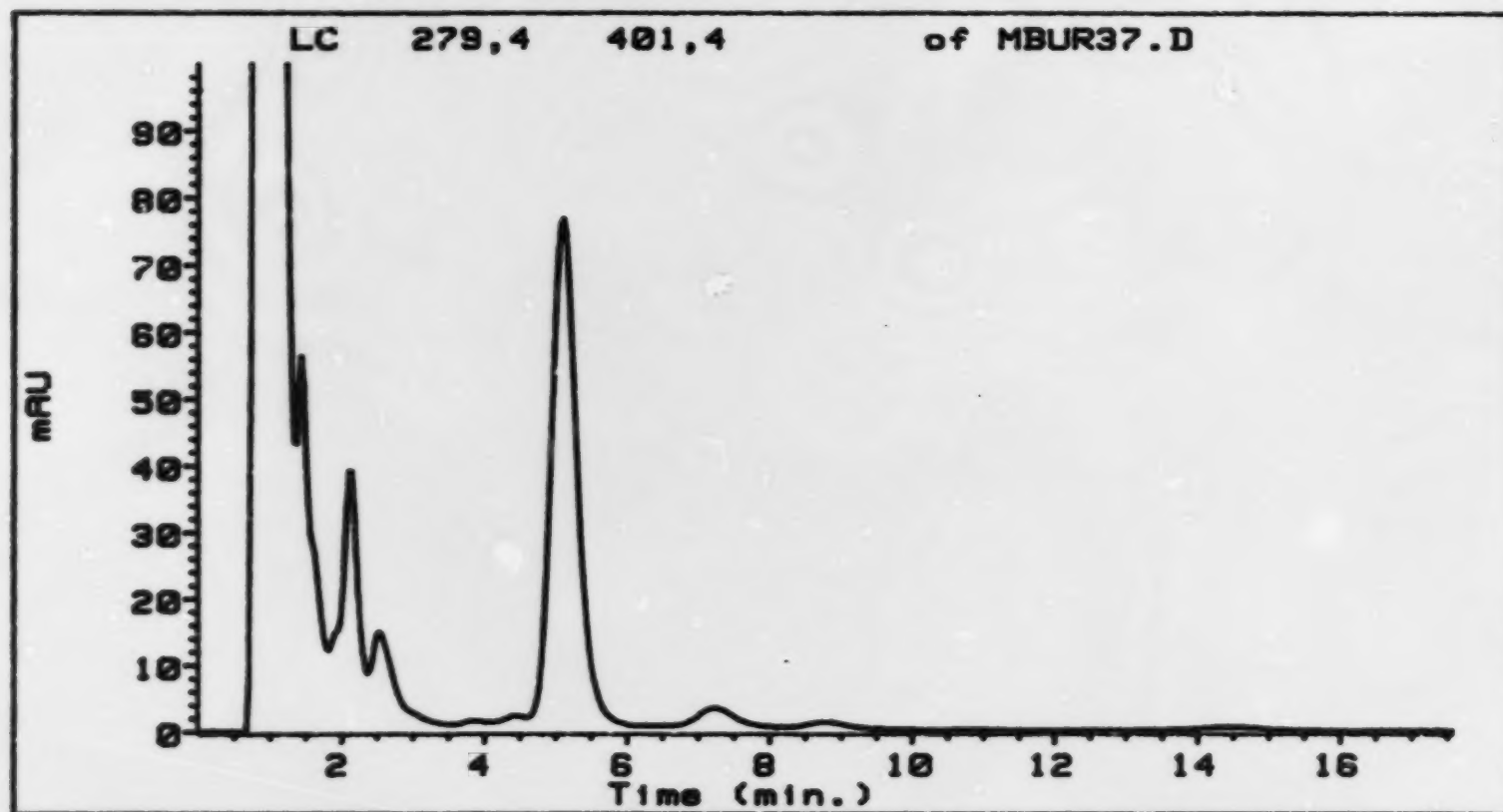


Fig. 5

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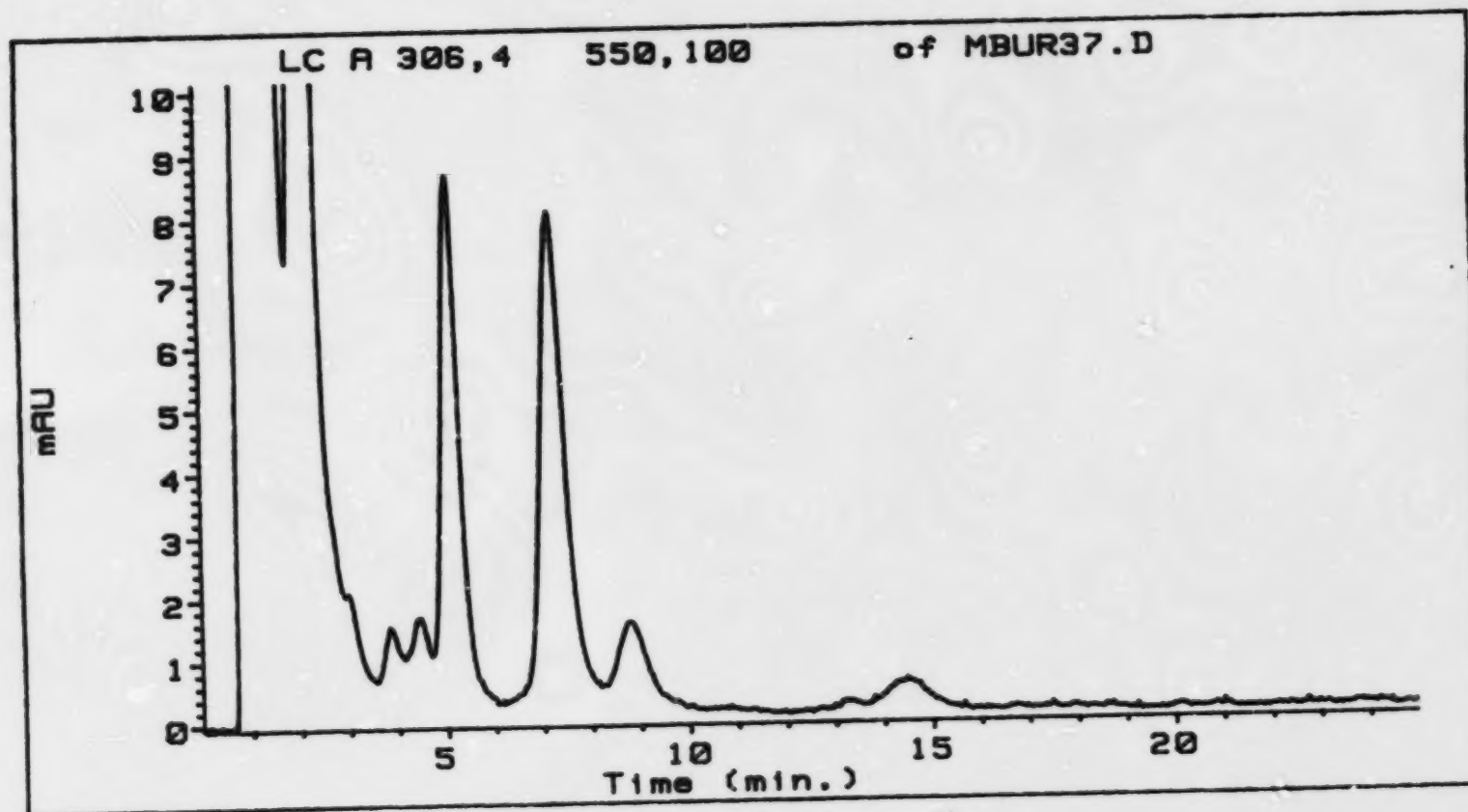


Fig. 6

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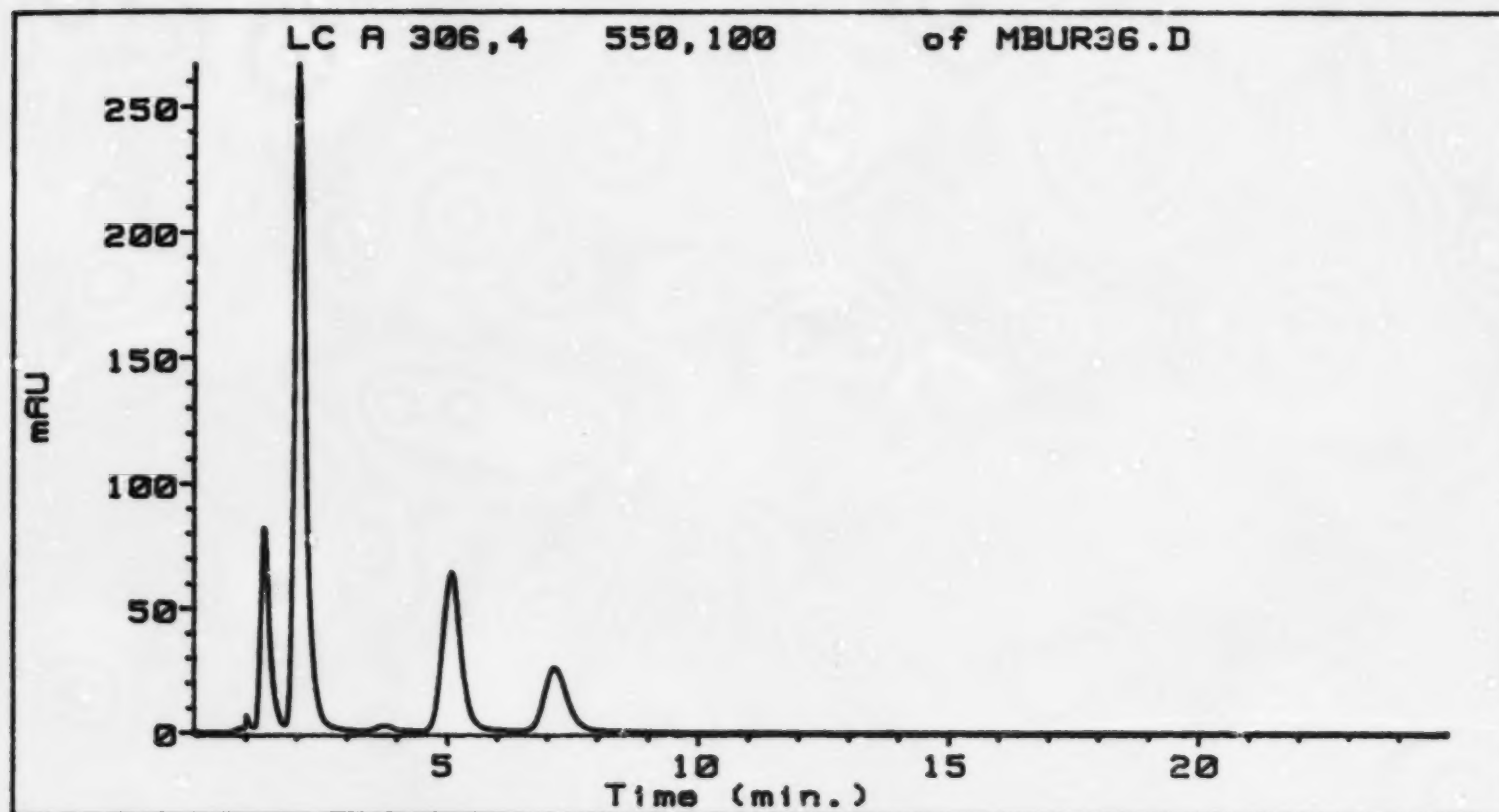


Fig. 7

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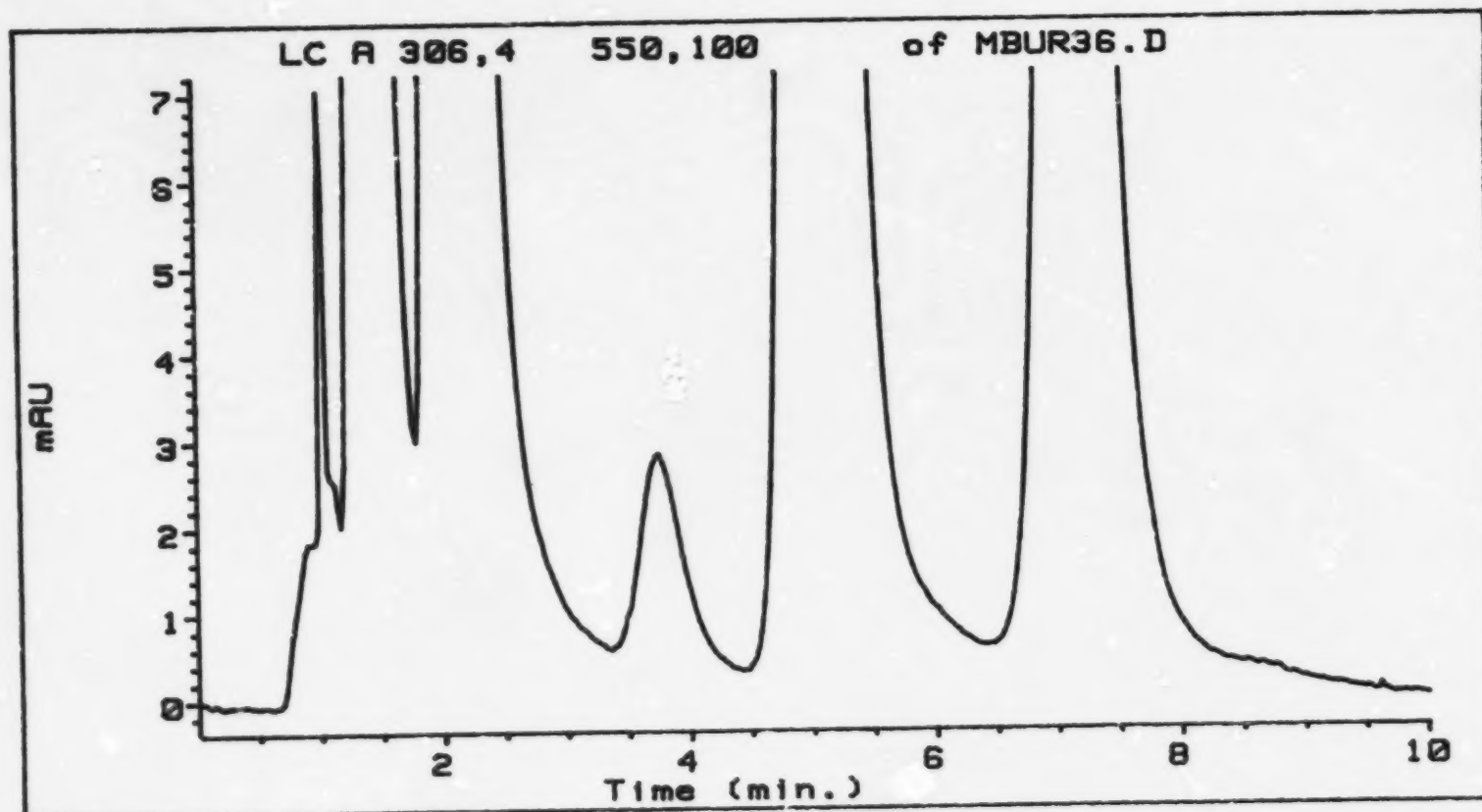


Fig. 8

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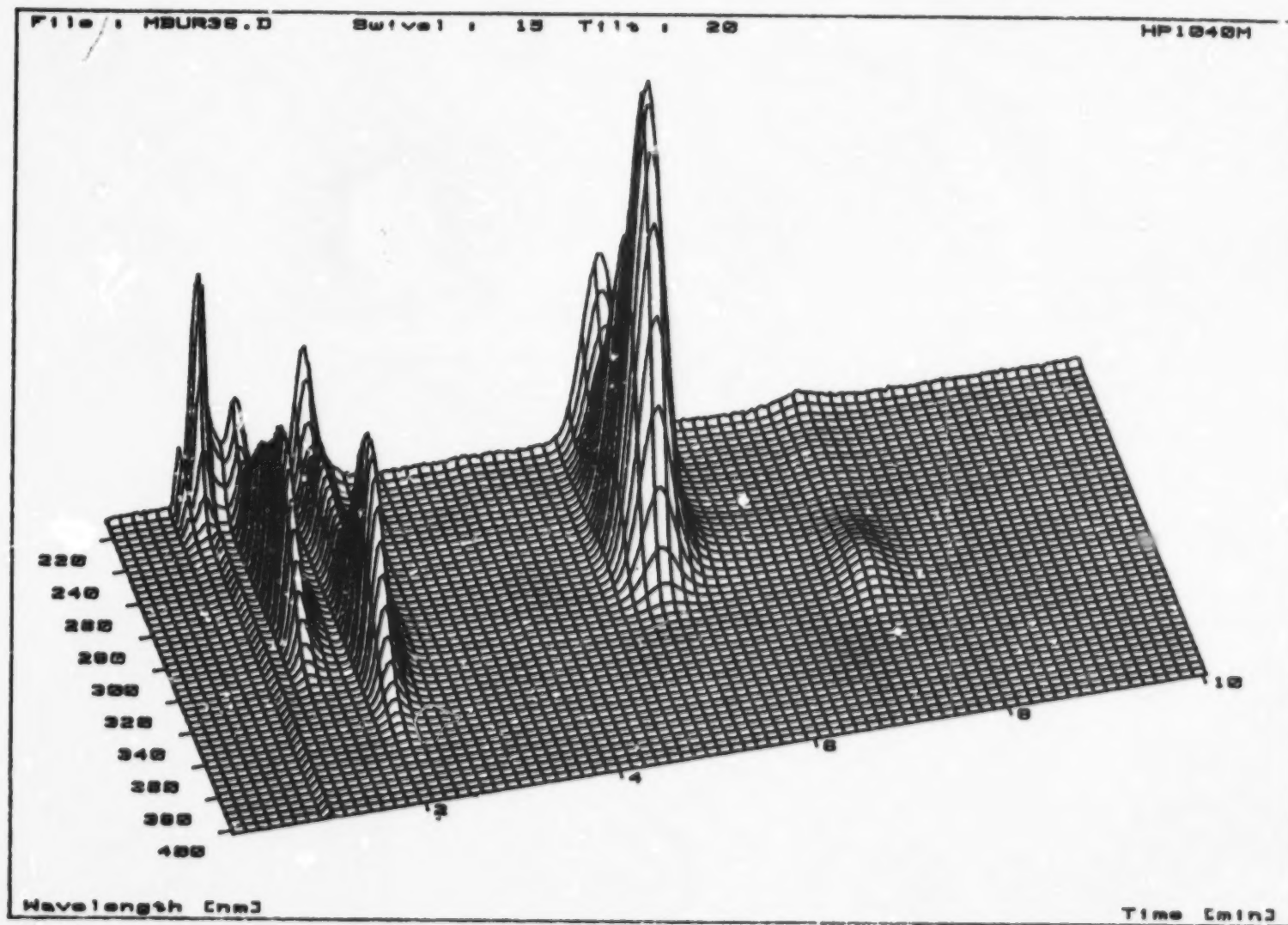


Fig. 9

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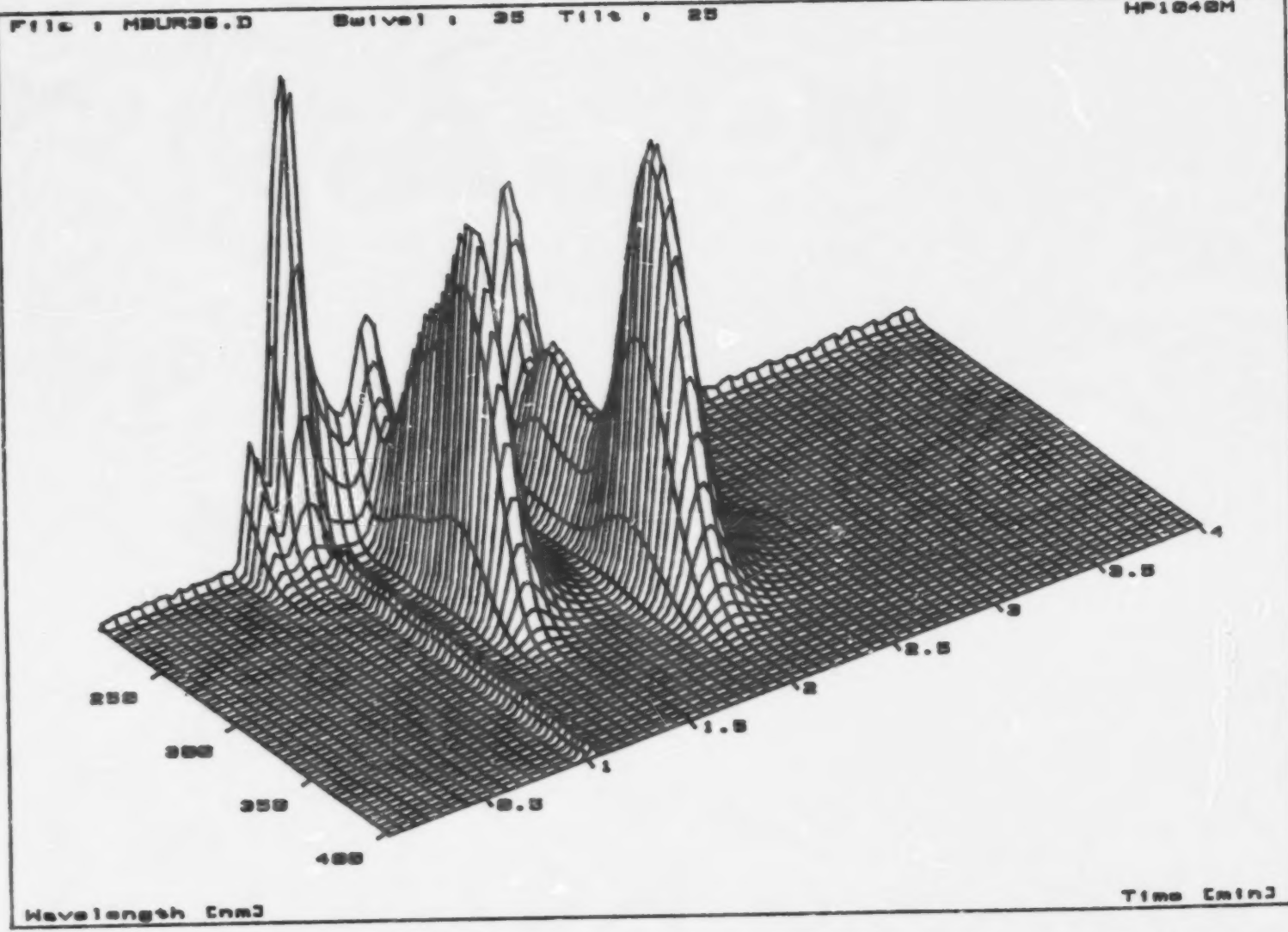


Fig. 10

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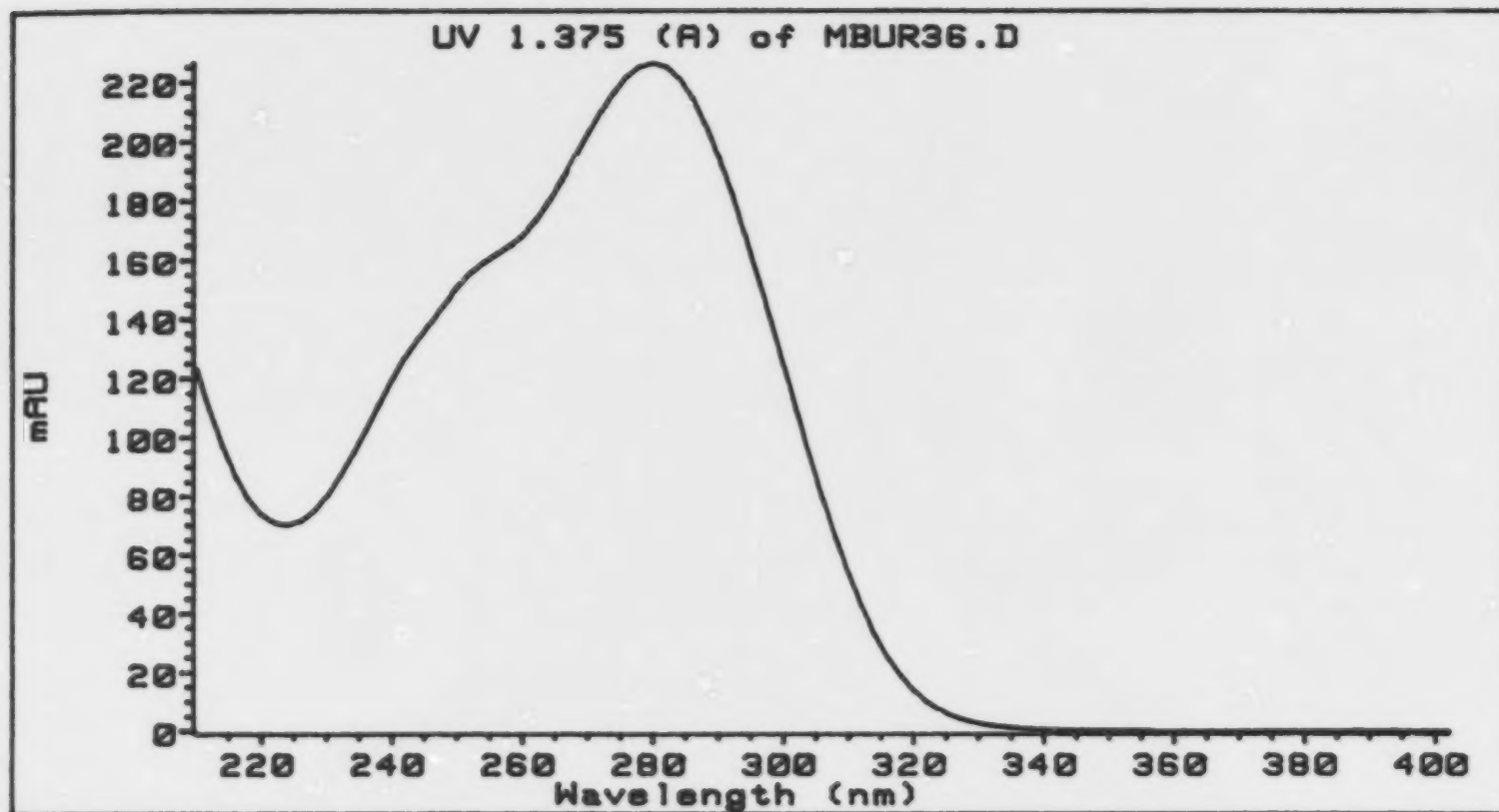


Fig. 11

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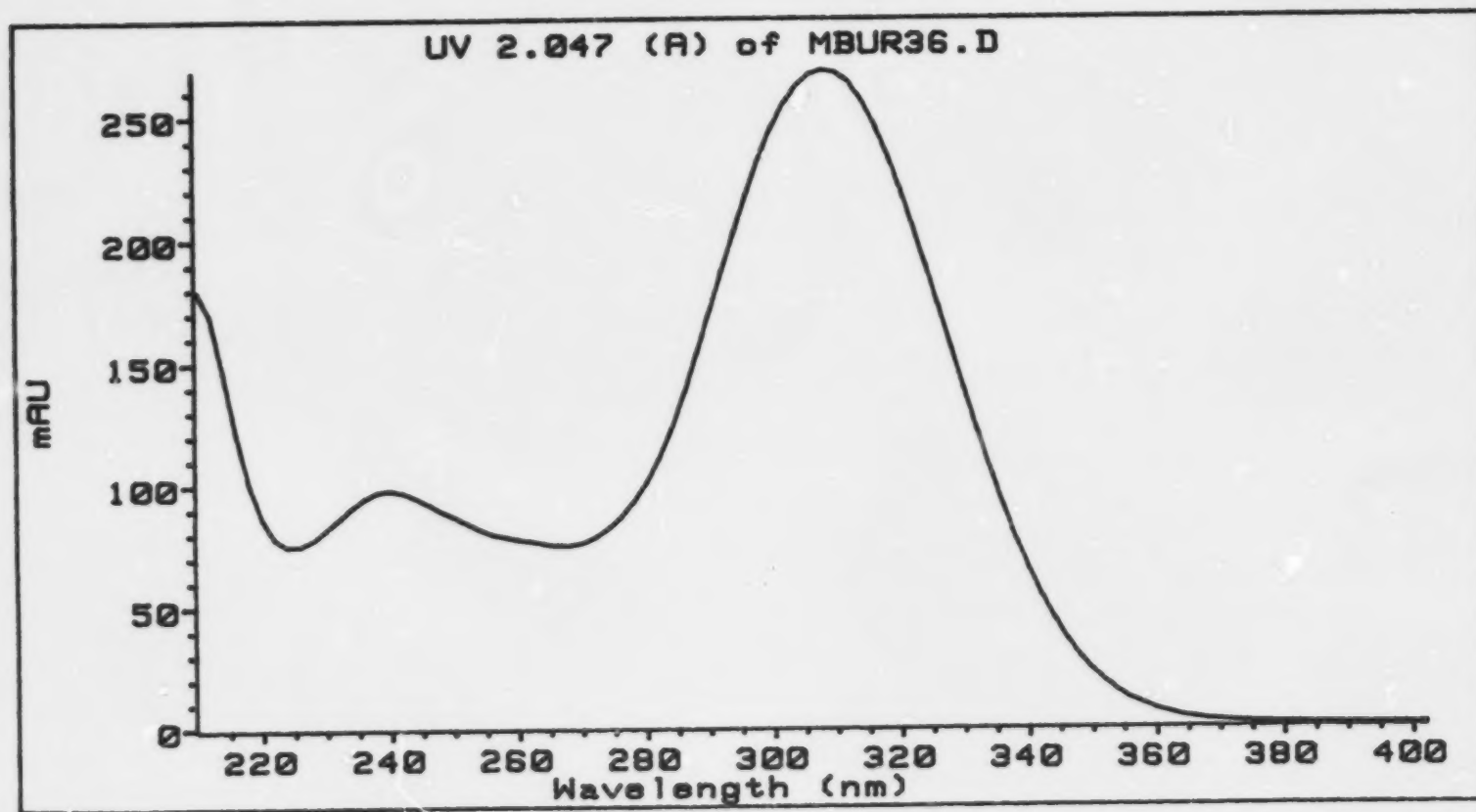


Fig. 12

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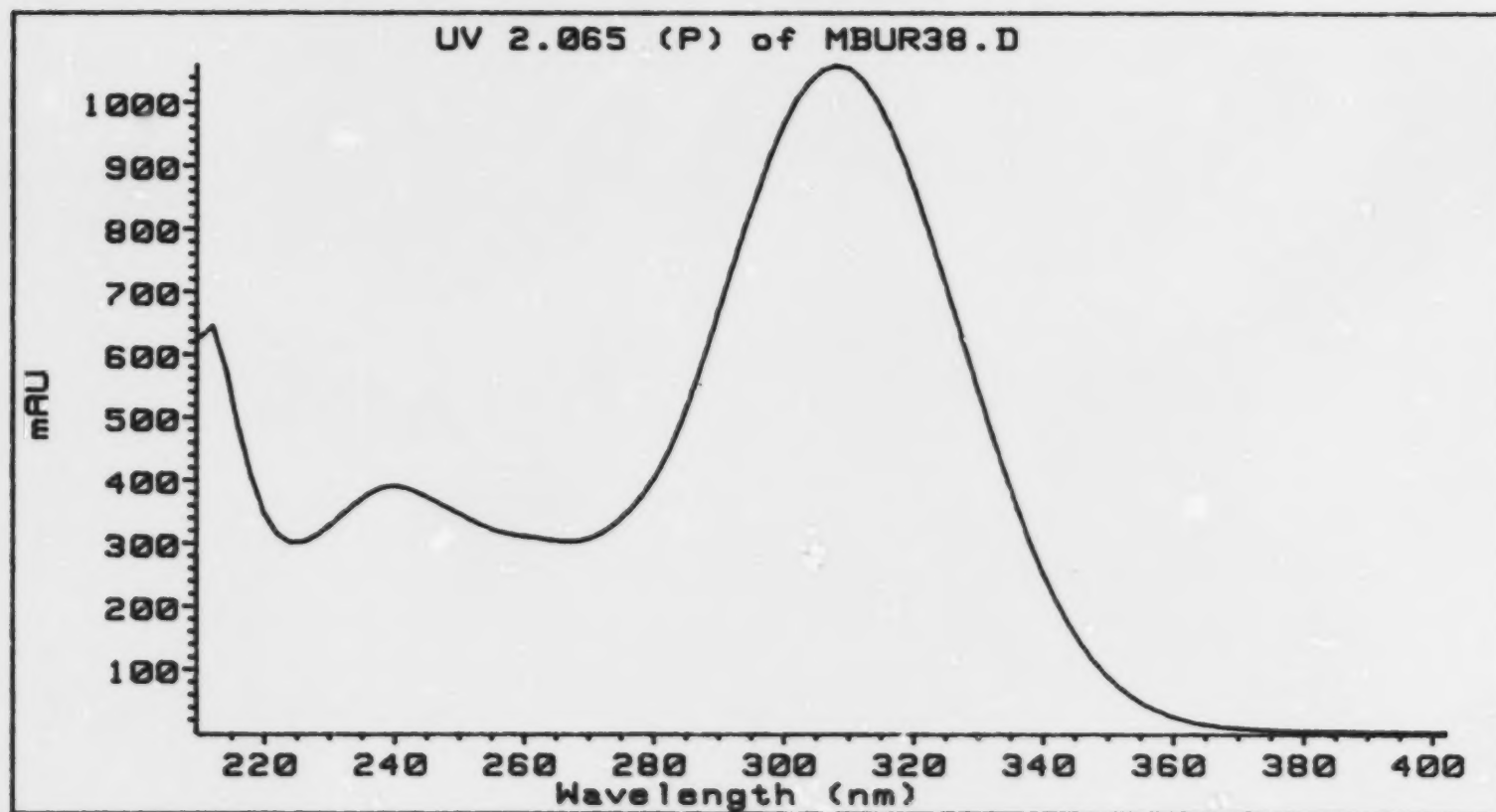


Fig. 13

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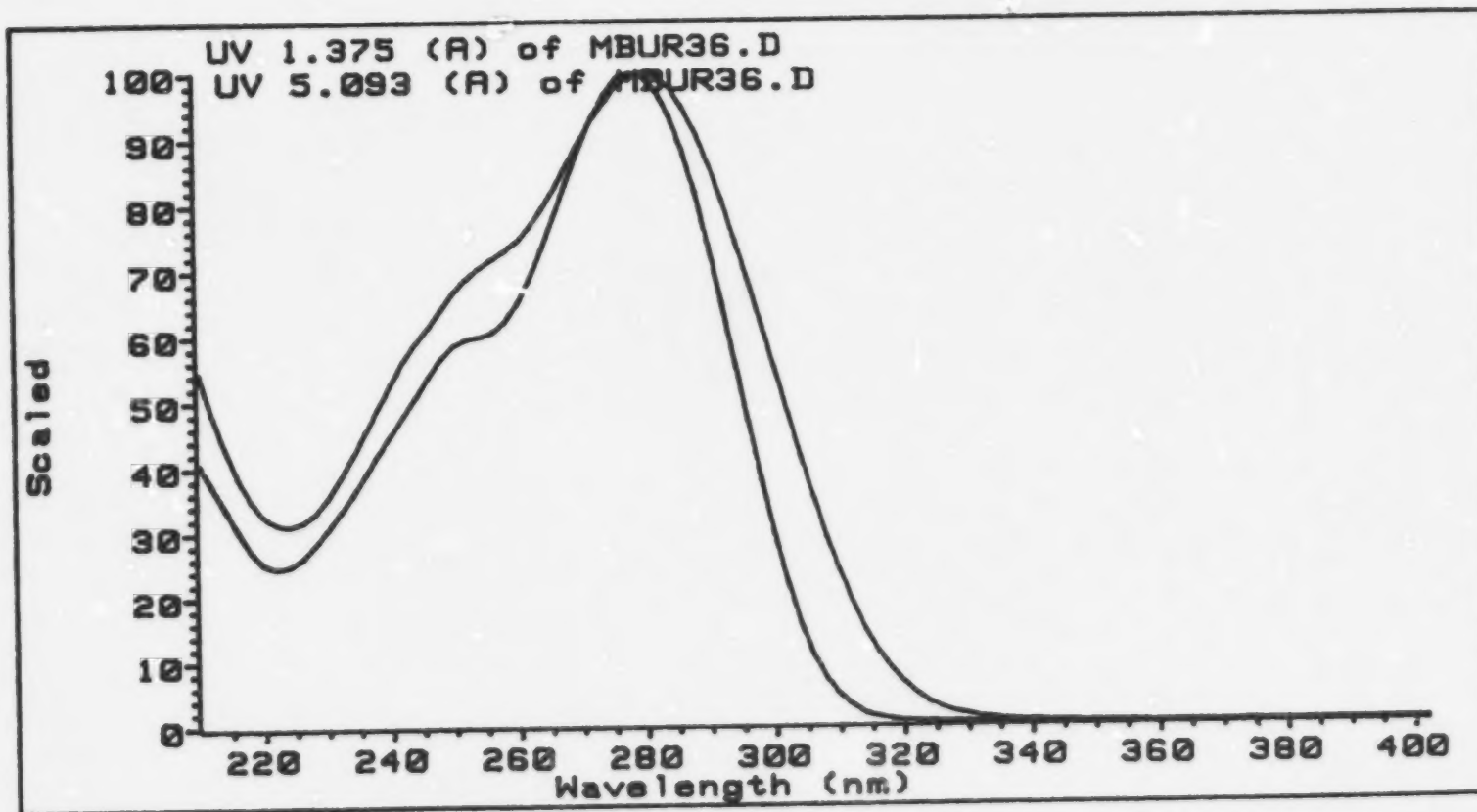


Fig. 14

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PHASE I AND CLINICAL PHARMACOKINETIC STUDIES ON MERBARONE
AT THE MEMORIAL SLOAN-KETTERING CANCER CENTER

November 18, 1986

R.P. Warrell, Jr., M.D., T.D. Walsh, M.D., C.W. Young, M.D.

INTRODUCTION

Merbarone was advanced to clinical trial because of its activity in rodent tumor model systems. The initial schedule chosen for clinical development was continuous infusion over 5 days. A continuous infusion program was chosen because a multiple daily injection schedule produced an improved therapeutic index and because the drug produced acute central nervous system toxicity in animals given bolus injections.

The early clinical trials, reported from MSKCC and from OSU, demonstrated: a) no biologic effect other than local phlebitis, and b) a slower rate of clearance than that of dogs, but no systemic toxicity at plasma levels of merbarone that would have been expected to be toxic in dogs.

The trials were continued with escalation steps of 200, 250, 300, 400, and 500 mg/m²/day x 5 days by continuous intravenous infusion. In addition, because it had become evident that the dog was not fully predictive of the drug's pharmacodynamic effects in humans, it was elected to initiate a 2-hour infusion schedule on a daily x 5 basis. For this the starting dose was reduced again to 150 mg/m²/day.

RESULTS

Three patients each have been enlisted in the trial at each of the following continuous infusion dosage levels: 100, 200, 250, 300, and 400 mg/m²/day x 5 days; one patient has completed therapy at the 500 mg/m² dosage level. Other than producing local phlebitis when the drug was administered into peripheral veins, this therapy has produced no consistent biologic effect. These data are presented in the accompanying Tables.

One patient has received treatment by the 2-hour infusion schedule; this produced local phlebitis at each peripheral infusion site but no other ill effect.

Detailed pharmacokinetic data are available at dosage levels of 100, 150, 200, and 300 mg/m²/day x 5 days. The results are presented in the accompanying Table. Within that dosage range there is no evidence of dose-dependent pharmacokinetic behavior.

Isolated steady state levels in one patient at 400 mg/m²/day were 18.2 and 19.6 ug/ml; in the one patient treated at 500 mg/m²/day levels of 52.7 and 57.9 ug/ml were measured. In view of the unexpectedly high levels in this patient, the remaining patients entered at this level will have full pharmacokinetic studies.

PHASE I TRIAL OF MERBARONE

TRIAL DESIGN

- DAILY x 5 DAYS
- INITIAL DOSE = 100 MG/M2/DAY BY CI
- NEW SCHEDULE=2 HR INFUSION AT 150 MG/M2/DAY
- REPEATED Q21-28 DAYS
- NO ESCALATION IN THE SOME PATIENT

MSKCC

CONCLUSIONS

1. When given by a central venous catheter, merbarone has been well tolerated; we have observed no consistent toxic effect in patients receiving up to 500 mg/m2/day x 5 days.

2. It appears unlikely that the drug can be administered by peripheral vein even with the 2 hour infusion schedule; however, we have observed no acute toxicity of the shorter infusion at the 150 mg/m2/day dosage.

3. The drug's pharmacokinetic behavior is essentially similar between the 100 and 300 mg/m2/day dosage levels; further data are required at the 500 mg/m2/day dosage.

PHASE I TRIAL OF MERBARONE
PATIENT CHARACTERISTICS

<u>HISTOLOGIC DIAGNOSES:</u>	<u>N=20</u>
LUNG (NSC)	7
KIDNEY	2
COLON	1
BLADDER	1
HEAD AND NECK	1
BREAST/GASTRIC	1
OVARY	1
PENIS	1
ENDOMETRIUM	1
SARCOMA	1
LYMPHOMA	2
THYMOMA	1
PERFORMANCE STATUS	70 (50-90)
AGE	50 (20-74)
PRIOR CHEMOTHERAPY	18/20

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DOSE ESCALATION BY CONTINUOUS INFUSION

<u>DOSE (MG/M²/D X 5)</u>	<u>PATIENTS ENTERED</u>	<u>PATIENTS EVALUABLE</u>	<u>PENDING EVALUATION</u>	<u>INEVALUABLE</u>
100	3	3	-	-
150	3	3	-	-
200	3	3	-	-
250	3	3	-	-
300	3	3	-	-
400	3	3	-	-
500	1	0	1	-

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Merbarone
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HEMATOLOGIC TOXICITY OF MERBARONE

<u>DOSE</u> <u>MG/M²/ X 5</u>	<u>WBC NADIR X 1000</u>		<u>PLATELET NADIR X 1000</u>	
	<u>MEDIAN</u>	<u>RANGE</u>	<u>MEDIAN</u>	<u>RANGE</u>
100	5.4	5.1-10.6	341	208-440
150	10.0	7.5-24.5	379	287-404
200	5.6	4.2- 9.9	275	230-410
250	7.4	6.4- 8.1	339	262-392
300	5.0	4.1- 7.0	269	114-319
400	11.1	7.5-18.1	222	215-344
500	--	--	--	--

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TOXICITY BY CONTINUOUS INFUSION

<u>DOSE</u> <u>(MG/M2/D x 5)</u>	<u>PHLEBITIS</u>	<u>VOMITING</u>	<u>DIARRHEA</u>	<u>WEAKNESS</u>	<u>CNS</u>
100	0/3	1/3	-	2/3	-
150	3/3	0/3	-	-	-
200	-/-	1/3	1/3	1/3	-
250	-/-	1/3	-	-	-
300	-/-	0/3	-	-	-
400	-/-	0/3	-	-	-
500	-/-	0/1	-	-	-

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PHARMACOKINETIC PARAMETERS DERIVED FROM EXPONENTIAL ANALYSIS
OF THE CONCENTRATION X TIME DATA FOR MERBARONE

<u>PATIENT #</u>	<u>DOSE</u> <u>MG/M2</u>	<u>CPSS</u> <u>UG/ML</u>	<u>AUC</u> <u>UG·HR/ML</u> <u>0-∞</u>	<u>CLTB</u> <u>ML/MIN/M2</u>	<u>MRT</u> <u>HR</u>	<u>VDss</u> <u>L/M2</u>	<u>α₁</u> <u>HR</u>	<u>α₂</u> <u>HR</u>
1	100	3.2	385	21.7	10.1	13.1	0.98	8.4
2	100	4.8	567	14.7	11.8	10.4	0.20	11.4
3	100	9.1	1078	7.7	9.2	4.3	1.0	7.5
4	150	6.7	815	15.3	20.5	18.8	5.8	37.4
5	150	10.3	1301	9.6	65.7	37.9	7.9	132
7	200	9.0	1046	15.9	6.4	6.1	4.4	--
8	200	16.7	2003	8.3	16.2	8.1	0.27	14.2
9	200	11.3	1340	12.4	11.1	8.3	3.5	17.5
13*	300	22.7	2690	9.3	9.9	5.5	6.9	--
14	300	18.7	2212	11.2	17.8	11.9	6.6	26.0
15**	300	11.4	1327	18.8	8.5	9.6	5.9	--

* DAY 6, 1ST SAMPLE AT 1 HR; ** DAY 6, 1ST SAMPLE AT 2 HR.

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PHASE I TRIAL OF MERBARONE

CONCLUSIONS

- 1) WELL-TOLERATED BY 5 DAY CONTINUOUS INFUSION WITH CENTRAL CATHETER.
- 2) NO MAJOR TOXICITY TO DOSE OF 500 MG/M²/D X 5.
- 3) NO EVIDENCE OF CUMULATIVE TOXICITY.
- 4) PHLEBITIS AT INJECTION SITE WITH DOSES $\geq$ 150 MG/M²/D
- 5) CURRENT FORMULATION CANNOT BE ADMINISTERED BY PERIPHERAL VEIN.

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6-TG daily x 5

Phase I Study

Eligibility Criteria

1. WBC $\geq$ 4,100 cell/mm³
2. Plts $\geq$ 130,000/m³
3. Hgb $\geq$ 10 g/dL
4. Normal direct reacting bilirubin
5. SGOT & alkaline phosphatase $\leq$ 3 x's normal
6. Creatinine $\leq$ 1.5
7. ECOG status 0-3

Patient Characteristics

Men	15
Women	21
Ages 25-70 years	
ECOG PS 0-1	26
2-3	10
Prior Chemo	Yes - 30
	No - 6
Prior RoRx	Yes - 5
	No - 31

Tumor Types

Colon	18
Adenocarcinoma UGI	5
Adenocarcinoma Unknown	2
Melanoma	4
Epithelial cancers	7

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Table 1 Hematological toxicity of 6-TG as a function of dose (initial course)

Daily dose (mg/m ²)	Patients	Median ^a nadir of cell count ($\times 10^3$ cells/ μ l)	
		Leukocytes	Platelets
15	3	5.8 (4.1-8.0)	234 (232-272)
30	3	3.5 (1.4-10.4)	148 (67-278)
37.5	3	2.5 (2.4-5.9)	95 (93-124)
45	3	3.7 (2.9-4.7)	108 (83-162)
50	3	3.7 (3.7-4.9)	140 (50-160)
55	15	3.8 (0.3-5.9)	150 (105-240)
65	6	2.2 (2.0-2.6)	80 (48-188)

^a Numbers in parentheses, range.

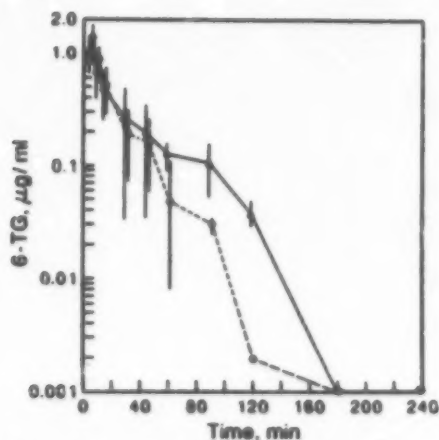


Fig. 1. Plasma 6-TG concentration after rapid i.v. infusion of 55 mg/m² on day 1 (C) and day 5 (Δ), shown as mean $\pm$ SD for 9 patients on day 1 and 6 patients on day 5.

No CR, PR

19 Stable - 16 patients with colon cancer
(1 - 8 courses)

1 squamous cell (1 cycle)

1 pancreas (1 cycle)

1 medullary thyroid cancer (5 cycles)

17 Progression

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Conclusion

Recommend dose of $55 \text{ mg/m}^2 \times 5 \text{ q } 5 \text{ weeks}$
for Phase II trials

6-TG by continous infusion
for 120 hours
Phase I Study

Patient Eligibility

WBC $\geq 4,100 \text{ cells/m}^3$

Platelets $\geq 130,000/\text{m}^3$

Hemoglobin $\geq 10 \text{ g/dl}$

Normal direct reacting serum bilirubin

Serum creatinine ≤ 1.5

ECOG P.S. 0-3

Pt characteristics - 9 pts.

Male 6 Female 3

Ages 39-67

ECOG PS	0-1	6
	2-3	3

Prior Chemotherapy 9

Prior Radiation Therapy 1

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Primary Sites

Colon	3
Breast	1
Pancreas	1
Skin-Melanoma	3
Skin - SCE	1

Hematologic Toxicity

Median Nadir x 10³
(Range)

Initial Course
mg/m²

<u>Daily x 5</u>	<u>Patients</u>	<u>Leukocytes</u>	<u>Platelets</u>
15	3	5.9 (3.4-10.9)	286 (153-300)
30	3	4.0 (2.5- 6.7)	114 (90-150)
37.5	3	1.6 (1.6- 1.8)	110 (20-140)

Non-Hematologic Toxicity

Response

Mild anorexia, nausea, vomiting

Headaches

Dermatitis

Stomatitis

No CR, PR

2 stable

7 progression

1 colon (2 cycles)
1 pancreas (1 cycle)

Conclusion

Recommend dose of $35 \text{ mg/m}^2 \times 5 \text{ q } 5 \text{ weeks}$
continuous infusion for
Phase II trials

IV 6-Thioguanine
T84-0513
Mayo Clinic
November 1986

INTRAVENOUS 6-THIOGUANINE IN
REFRACTORY ACUTE LEUKEMIA (AL)

- RATIONAL: 1) Erratic oral absorption
- 2) *Two randomized prospective trials found no advantage to triple combination of Anthracycline, ARA-C, and 6-TG over Anthracycline, ARA-C in AL patients

PLAN: Daily 10 minute infusion 6-TG x 7 days

Dose escalation 60 to 300 mg/m²

Frequency: 15 to 21 days

*Scan Journ Hemat, Vol. 23, p. 124, 1979.
Blood, Vol. 62, No. 5, Suppl. 1, p. 206, 1983

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ELIGIBILITY

Refractory acute leukemia (ANLL, ALL, BL-CGL)

Performance score 0-2

No active infection

No chemotherapy for at least two weeks (except HU)

Creatinine < 2.0 m/gdl

Total bilirubin < 2.0 mg/dl

PTS CHARACTERISTICS

Male 8 Female 2

Mean Age (50) Range 24-73

60 mg/m ²	3 courses
75 mg/m ²	2 courses
90 mg/m ²	5 courses (4 initial treatment)
135 mg/m ²	4 courses (4 retreatment)
200 mg/m ²	6 courses (4 retreatment)
300 mg/m ²	3 courses (2 retreatment)

TOXICITY

Gastrointestinal: Nausea, mild - not dose related

No vomiting

Hepatic: Mild elevation LFT usually caused
by infection (no VOD noted)

Renal: No toxicity

Cutaneous: Photosensitivity

Hyperpigmentation

Stomatitis: Dose related - seen only at ≥ 200 mg/m²
x 7 days

1 pt unusually severe mucositis

Normal Thiopurine Methyltransferase
level

IV 6-Thioguanine
T84-0513
Mayo Clinic
November 1986

RESPONSE

No CR or PR

1 pt with CGL had regression of splenomegaly
from 20+ cm to 3 cm x 5 months

5 pts with WBC > 10,000 developed leukopenia
< 2,000 without marrow remission

Onset of myelosuppression never
before completing D7 injection

CONCLUSION

Intravenous 6-Thioguanine can be given
safely at dosage as high as $200 \text{ mg/m}^2 \times 7 \text{ days}$

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PHASE I AND CLINICAL PHARMACOKINETIC STUDIES ON HMBA
AT THE MEMORIAL SLOAN-KETTERING CANCER CENTER

November 18, 1986

M.P. Fanucchi, M.D., T.D. Walsh, M.D., C.W. Young, M.D.

INTRODUCTION

The MSKCC Phase I study was initiated as an extension of the 5-day continuous infusion studies recently completed at the U. of Maryland Cancer Center and at the Johns Hopkins Hospital. It is based upon the same design philosophy as that of the previous studies, i.e. to maintain a steady concentration of HMBA in plasma over a period of days, in the hope of producing antitumor effects. The present study employs a 10-day continuous infusion; courses are repeated at 28 to 42 day intervals. This study is to be followed by disease-oriented studies both in melanoma and RAEB. The content of HMBA is being measured by gas chromatography using a capillary column.

RESULTS

Eighteen patients have been enlisted in the study at dosage levels of 12, 16, and 20 gm/m²/day x 10 days. The patient characteristics and the observed effects of the drug are presented in the accompanying Tables. The principal toxic effect of the drug has been thrombocytopenia. Platelet count depression has been observed at all dosage levels; a clear dose-effect relationship has not yet been established. The most severe platelet depressions have occurred in patients with disease-related sites of platelet consumption. Other than the above-mentioned thrombocytopenia, we have observed no consistent toxic effect.

We have observed stabilization of pulmonary metastases in one patient receiving repeated courses of therapy for over 11 months. There has been no other evidence of anticancer effect.

The steady state plasma concentrations at the 12 and 16 gm/m² dosage levels, and the pharmacokinetic parameters from compartmental analysis of 4 patients at the 16 gm/m² dosage level are provided in the accompanying Tables. The mean steady state for the group increased from 0.45mM at 12 gm/m²/day to 0.66mM at 16 gm/m²/day. The terminal clearance of the drug fit a 1 compartment model.

CONCLUSIONS

1. Over the dosage range 12 to 20 gm/m²/day by continuous infusion, HMBA has been well tolerated; the only consistent effect has been thrombocytopenia.

2. Although individual patients have experienced significant thrombocytopenia, we have not yet reached the MTD for the group. In consequence we plan to proceed to the 24 gm/m²/day dosage level.

HMBA
T85-0222
MSKCC
November 1986

PHASE I TRIAL OF HMBA

STUDY DESIGN

- DAILY X 10 DAYS - CONTINUOUS INFUSION
- DOSE LEVELS: 12, 16, 20, 24GM/M2/DAY
- REPEATED Q 28-42 DAYS

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PHASE I TRIAL OF HMBA
PATIENT CHARACTERISTICS

Age: Median 56.5 Range 38-69

KPS: Median 70% Range 60-100%

Sex: Male 13 Female 5

Prior Rx: None 4, Chemo 7, Chemo+RT 7

Diagnoses: NSC Lung Ca 4

 Colorectal Ca 3

 Esophageal Ca 2

 Leiomyosarcoma 2

 Melanoma 1

 Adenoca: 1 each from Breast,
 Stomach, Bile Duct, Kidney,
 Pancreas, and unk. primary.

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HMBA
T85-0222
MSKCC
November 1986

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HMBA
T85-0222
MSKCC
November 1986

PHASE I TRIAL OF HMBA
HEMATOPOIETIC TOXICITY

<u>Dosage</u>	<u>No of Pats</u>	<u>No of Courses</u>	<u>WBC Median</u>	<u>Nadir Range</u>	<u>Platelet Median</u>	<u>Nadir Range</u>
12 gm	6	13	4.4	2.1-7.7	146	53-245
16 gm	8	13	4.7	3.6-9.3	114	70-226
20 gm	7	10	5.6	2.8-10.0	131	11-189

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PHASE I TRIAL OF HMBA
NON-HEMATOPOIETIC TOXICITY

<u>Dosage</u>	<u>No of Pts</u>	<u>No of Courses</u>	<u>CNS</u>	<u>No. and Grade Observed</u>		
				<u>N+V</u>	<u>Renal</u>	<u>Diarrhea</u>
12 gm	6	13	1+	1+, 1+	1+, 2+	0
16 gm	8	13	0	2+, 2+	1+, 1+, 1+	3+
20 gm	7	10	0	0	0	1+

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PHASE I TRIAL OF HMBA
STEADY STATE PLASMA CONCENTRATIONS

<u>PATIENT</u>	<u>DOSAGE</u> <u>GM/M2/DAY</u>	<u>NO</u> <u>SAMPLES</u>	<u>MEAN HMBA</u> <u>MM</u>	<u>OBSERVED</u> <u>RANGE</u>
1	12	18	0.48 $\pm$ 0.17*	0.25 TO 1.5
2	12	15	0.30 $\pm$ 0.1	0.21 TO 0.49
3	12	17	0.24 $\pm$ 0.21	0.16 TO 1.8
4	12	18	0.88 $\pm$ 0.05	0.44 TO 1.9
5	12	11	0.52 $\pm$ 0.11	0.36 TO 0.61
6	12	11	0.27 $\pm$ 0.11	0.18 TO 0.35

* STANDARD DEVIATION

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HMBA
T85-0222
MSKCC
November 1986

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PHASE I TRIAL OF HMBA
STEADY STATE PLASMA CONCENTRATIONS

<u>PATIENT</u>	<u>DOSAGE</u> <u>GM/M2/DAY</u>	<u>NO</u> <u>SAMPLES</u>	<u>MEAN HMBA</u> <u>MM</u>	<u>OBSERVED</u> <u>RANGE</u>
5	16	7	0.73 $\pm$ 0.05*	0.72 TO 0.80
6	16	9	0.60 $\pm$ 0.05	0.53 TO 0.72
7	16	15	0.61 $\pm$ 0.08	0.51 TO 1.04
8	16	15	0.98 $\pm$ 0.12	0.79 TO 2.4
9	16	8	0.76 $\pm$ 0.11	0.6 TO 0.92
11	16	12	0.39 $\pm$ 0.07	0.25 TO 0.52
12	16	15	0.53 $\pm$ 0.07	0.44 TO 0.62

* STANDARD DEVIATION

MSKCC

PHARMACOKINETIC PARAMETERS HMBA*

PATIENT	CP _{SS}	CP (MAX)	AUC _{0-∞}	MRT	CL _{TB}	VD _{SS}	HALF-LIFE
	<u>MM</u>	<u>MM</u>	<u>MM·HR</u>	<u>HR</u>	<u>ML/MIN/M2</u>	<u>L/M2</u>	<u>HR</u>
7	0.57	0.76	122	3.4	109	22.1	2.3
8	0.86	1.31	201	3.6	66	14.3	2.5
11	0.30	0.52	73	0.9	182	9.3	0.6
12	0.47	0.62	113	2.5	118	17.8	1.7

*DOSAGE: 16GM/M2/DAY X 10 CONTIN. I.V. INFUSION

MSKCC

PHASE I TRIAL OF HMBA

INTERIM SUMMARY

- HMBA RELIABLY PRODUCES THROMBOCYTOPENIA
- 20GM/M2/DAY X 10 IS NOT THE MTD
- ONE PATIENT HAS EXPERIENCED DISEASE STABILIZATION
- WE WILL PROCEED TO THE 24GM/M2/DAY LEVEL

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HMBA
T85-0222
MSKCC
November 1986

301

PHASE I STUDY OF HMBA

THE JOHNS HOPKINS ONCOLOGY CENTER

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Five-Day Study

In our initial phase I study of HMBA administered as a continuous five-day infusion every three weeks, toxicities consisting of renal insufficiency, a hyperchloremic metabolic acidemia/acidosis and central nervous system toxicity, manifested by agitation and delirium, were dose-limiting toxicities. Mild myelosuppression, mucositis, nausea and vomiting were also observed. Based on this trial, the maximum-tolerated (MTD) and recommended phase II doses were 33.6 and 24 g/M²/day respectively.

HMBA concentrations in plasma and urine were measured by gas chromatography using octamethylene bisacetamide (OMBA) as an internal standard (Table 1). Steady-state was obtained relatively rapidly and drug accumulation over time was not observed. Mean steady-state concentrations (C_{ss}) were 0.88 ± mM at 15.8 g/M²/day, 0.98 ± 0.16mM at 24.0 g/M²/day, and 1.58 ± 0.17 mM at 33.6 g/M²/day. These concentrations only approached the lower limits demonstrated for in vitro differentiating

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effectiveness (1-5mM for HL-60 promyelocytic and murine erythroleukemic cells [MEL]). CNS toxicity was observed in the two oldest patients on study who also developed concomitant renal toxicity with HMBA Ccs approaching 2mM. Eleven of 12 HMBA courses with mean HMBA Ccs > 1mM developed acidemia which was always associated with a worsening of renal function.

The pharmacokinetics of HMBA best fit a single compartmental model. Table 2 demonstrates several pharmacokinetic parameters and curves derived from nonlinear least square analysis of plasma concentrations. Large variations among patients were obtained including half-lives of elimination which ranged from 8 to 301 minutes (mean, 144 min). The apparent volume of distribution was between plasma volume and total body water for the majority of patients and ranged from 1.1 to 30.2 L/M². Renal clearance ranged from 6 to 91 ml/min/M² (mean, 43 ± 6 ml/min/M²) and accounted for 15 to 70 percent (mean, 45 ± 6 percent) of the parent compound.

The apparent main metabolite of HMBA, 6-acetoamidohexanoic acid (Figure 1), was measured after derivitization with diazomethane. This metabolite undergoes similar renal disposition with a Cl_r ranging from 12 to 37 ml/min/M² (mean, 28 ± 7 ml/min/M²). Mean steady-state 6-acetoamidohexanoic acid concentrations were 1.44 ± 0.60mM (range 0.46 to 2.90mM) at 24 g/M²/day and 1.65 ± 0.29 (range, 1.15 to 2.40mM) at 33.6 g/M²/day. The small anion gap observed with HMBA at dose of 24 and 33.6 g/M²/day can be potentially explained by concentrations of this

metabolite in the range of 1 to 3mM. Renal excretion of HMbA and of the 6-acetamidohexanoic acid metabolite, together account for the disposition of 66 to 93 (mean, 74 ± 6) percent of the infused drug.

Ten-Day Study

Since steady-state HMbA levels at the MTD and recommended phase II doses during the five-day infusion study were lower than desirable, and because of evidence suggesting that the exposure period may be an important factor in the induction of differentiation, an additional phase I study examining a longer ten-day continuous infusion schedule was begun.

In our most recent phase I study of HMbA, the drug was administered as a continuous infusion for ten consecutive days of a 28-day cycle. Our starting dose was $15.8 \text{ g/m}^2/\text{day}$ since only minor toxicity was observed at this dose level during our initial five-day study. If tolerated, we planned to escalate the dose to $20 \text{ g/m}^2/\text{day}$, and subsequently to $24.0 \text{ g/m}^2/\text{day}$, if appropriate. Thus far, 12 patients received 17 courses of HMbA as a ten-day infusion. Patient characteristics are shown in Table 3. The number of patients and courses for each dose escalation is shown in Table 4. A summary of toxicity by ECOG criteria at all dose levels appears in Table 5.

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In contrast to our initial five-day study where myelosuppression was mild, myelotoxicity, primarily thrombocytopenia, was the dose-limiting toxicity during our ten-day study. All 17 courses were associated with an absolute decrease in the platelet count. Table 6 demonstrates the effects of HMBA on platelet counts. At the starting dose, $15.8 \text{ g/M}^2/\text{day}$, 3 courses were associated with ECOG grades 1 and 2 thrombocytopenia, and 1 course was associated with ECOG grade 4 thrombocytopenia. Grade 4 thrombocytopenia at $15.8 \text{ g/M}^2/\text{day}$ occurred in a 62-year old male with stage 4 diffuse histocytic lymphoma who received the heaviest pretreatment chemotherapy and radiotherapy among the study patients. He received a second course of HMBA at $12 \text{ g/M}^2/\text{day}$, but the infusion was stopped on day 8 due to his platelet count which had decreased to 55,000/ul, and subsequently, nadired at 46,000/ul. Four of the six courses of HMBA administered at $20 \text{ g/M}^2/\text{day}$ resulted in grades 3 and 4 thrombocytopenia; the remaining 2 courses were associated with grades 1 and 2 thrombocytopenia. It should be noted that the nadir platelet count occurred approximately on days 13 through 18, with recovery generally by days 22 through 29. Bone marrow examinations during thrombocytopenia have demonstrated scant numbers of megakaryocytes. Significant treatment-related leukopenia and anemia occurred less frequently than thrombocytopenia. At the $15.8 \text{ g/M}^2/\text{day}$ dosage level, 2 of 10 courses were associated with grades 1 and 2 leukopenia. At the $20 \text{ g/M}^2/\text{d}$ dosage level, 4 of 6 courses were associated with grades 1 and 2 leukopenia and one patient developed grade 4 leukopenia. Leukopenia and anemia generally

occurred in patients who had the most significant thrombocytopenia. Heavy prior treatment with chemotherapy and radiotherapy, especially pelvic irradiation, increased the risk of developing myelotoxicity. Myelosuppression also tended to be cumulative -- nadir platelet and white blood counts were always lower following a patient's second course of HMBA.

Non-hematologic toxicities were similar to those observed during the shorter five-day HMBA infusions and are summarized in Table 7. Grades 1 and 2 renal toxicity and hyperchloremic metabolic acidemia ($\text{HCO}_3^- \leq 20$ meq/L) occurred in 3 of 10 courses at $15.8 \text{ g/M}^2/\text{day}$ and 2 of 6 courses at $20 \text{ g/M}^2/\text{day}$. Azotemia and acidemia were most pronounced towards the end of the HMBA infusion and rapidly resolved following drug discontinuation. Hyponatremia due to well-documented SIADH was noted during one course at $15.8 \text{ g/M}^2/\text{day}$, which also rapidly resolved following the discontinuation of the infusion. Infrequent nausea and vomiting, mucositis, anorexia, local cellulitis, and grade 1 hepatitis were also observed. Interestingly, 7 courses were associated with moderate leukocytosis which occurred both during and following the HMBA infusion. However, CNS toxicity was not observed, although 6 of 14 posttreatment EEGs developed changes consistent with mild diffuse disturbances of cerebral function.

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HMBA-mediated dermatologic toxicity was seen at the $15.8 \text{ g/M}^2/\text{day}$ dosage level. It occurred in a 65-year old female with a leiomyosarcoma of the jejunum with metastases to the liver. Past treatment included resection of the jejunum, doxorubicin, and trimetrexate. On day 12 following the first course of HMBA, the patient developed a periorbital and malar erythematous rash which intensified until day 15 and resolved by day 22 without desquamation. An ANA was positive at 1:40 (homogenous). During the second course of HMBA, the patient developed palpable purpura involving her ankles on day 3, which progressed proximally to her thighs by day 9. She also redeveloped the facial skin rash on day 10 which resolved by day 22. The infusion was discontinued on day 9 with resolution of the extremity rash by day 22. There was no evidence of extradermal vasculitis. An ANA titer was positive at 1:80, while serum C_3 , C_4 , and immune complexes were normal. A biopsy of the skin lesion revealed a leukocytocytoclastic vasculitis with perivascular deposition of IgA and C_3 .

Mean steady-state HMBA plasma concentrations (C_{ss}) and clearances are depicted in Table 8. Mean HMBA C_{ss} values were $0.75 \pm 0.14 \text{ mM}$ (range 0.35 to 1.68 mM) at $15.8 \text{ g/M}^2/\text{day}$ and $0.99 \pm 0.22 \text{ mM}$ (range 0.31 to 1.72 mM) at $20 \text{ g/M}^2/\text{day}$. Higher HMBA C_{ss} were observed in patients who developed renal toxicity. However, drug accumulation over time was generally not noted. As demonstrated in our initial five-day study, the primary metabolite in serum was 6-acetamidohexanoic acid. This metabolite is currently being quantitated.

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Lymphocyte levels of the polyamines — putrescine, spermidine, and spermine were measured prior to and during several HMBA infusions by cell lysis, dansylation, and HPLC. The rationale for polyamine measurements were threefold: 1) toxicities, specifically thrombocytopenia, resembled those associated with the administration of α -difluoromethyl-ornithine (DFMO), a potent inhibitor of the enzyme ornithine decarboxylase, which is essential for normal polyamine biosynthesis; 2) modulation of polyamines has been implicated as a possible mechanism for polar-planar solvent-induced differentiation; and 3) the structure of HMBA resembles the structure of several polyamines, especially putrescine. We could not discern any specific patterns of polyamine behavior during HMBA infusions, nor could we identify consistent relationships between polyamine changes and toxicity. Interestingly, levels of spermidine and spermine decreased to 10 and 33 percent of the initial levels, respectively, during the course of HMBA infusion that was administered to the 62-year old male with DEL. He was the most sensitive of our patients with respect to the myelotoxic effects of HMBA. In the course of our analysis of lymphocyte polyamine levels, we were able to identify two intracellular metabolites. These metabolites dansylate in contrast to HMBA or 6-acetoamidohexanoic acid. Structural analysis of these metabolites by mass spectroscopy is ongoing.

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No objective antitumor responses have been seen following the administration of HMBA as a ten-day infusion.

In summary, the toxicities of HMBA are schedule-dependent. Thrombocytopenia is the primary dose-limiting toxicity of HMBA when administered by a ten-day continuous infusion. When given by this schedule, the MTD and recommended phase II doses are 20 and 15.8 g/m²/day, respectively. Since steady-state HMBA concentrations at these dose levels were generally lower than those obtained when HMBA is administered for five days at the recommended phase II five-day dose, 24 g/m²/day, we do not think that the ten-day schedule offers any significant advantages over to the five-day schedule. The administration of HMBA for ten days may also be disadvantageous when administered to patients with myelodysplastic syndromes who often have depressed pretreatment platelet counts.

CONCLUSIONS

- o Thrombocytopenia is the dose-limiting toxicity of HMBA administered as a ten-day infusion.
- o The MTD and recommended phase II doses of HMBA on this schedule are 20.0 and 15.8 g/m²/d.
- o The mean steady-state plasma HMBA concentration at 15.8 g/m²/d is 0.75 nM.
- o HMBA administered as a ten-day infusion probably offers no significant advantage over the five-day infusion schedule.

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TABLE 1
HMBA STEADY-STATE CONCENTRATION CLEARANCE
(X 5 DAYS)

Dose (g/m ² /d)	Mean Steady-State HMBA Concentration (range) (ng)	Mean Systemic Clearance (range) ₂ (cc/min/m ²)	Mean Renal Clearance (range) ₂ (cc/min/m ²)	Mean Systemic/Renal Clearance
15.0	0.70 (0.34-0.97)	90 (57-102)	65 (35-57)	0.51
15.0	0.71 (0.59-0.85)	79 (65-95)	-	-
15.0	1.50	37	6 (5-6)	0.15
15.0	0.61 (0.53-0.68)	91 (81-100)	42 (32-51)	0.64
15.0*	0.88 ± 0.10	74 ± 13	31 ± 13	0.37 ± 0.11
24.0	0.53 (0.49-0.56)	150 (145-170)	74 (41-91)	0.51
24.0	0.47 (0.32-0.60)	105 (139-200)	-	-
24.0	0.52 (0.48-0.63)	135 (132-139)	72 (60-89)	0.59
24.0	0.69 (0.63-0.76)	121 (110-132)	52 (43-61)	0.65
24.0	1.34 (0.82-1.63)	66 (50-102)	18 (15-20)	0.33
24.0*	1.90	40	7 (6-9)	0.32
24.0	0.44 (0.33-0.52)	193 (160-203)	91 (54-98)	0.51
24.0	0.73 (0.54-0.88)	115 (95-150)	80 (57-107)	0.70
24.0	0.82 (0.84-0.94)	95 (80-99)	56 (44-66)	0.51
24.0	2.30 (1.82-2.50)	38 (35-46)	13 (10-17)	0.37
24.0	1.29 (1.04-1.44)	65 (51-88)	24 (11-30)	0.63
24.0	1.35 (1.02-1.54)	63 (54-82)	35 (10-50)	0.95
24.0*	0.98 ± 0.14	107 ± 16	48 ± 9	0.67 ± 0.03
33.0	1.10 (1.00-1.16)	76 (72-85)	47 (30-56)	0.61
33.0	1.35 (1.11-1.46)	87 (72-100)	55 (40-69)	0.62
33.0	1.72 (1.37-2.00)	69 (62-85)	-	-
33.0	2.09 (1.70-2.30)	57 (40-69)	19 (15-26)	0.37
33.0*	1.66 (1.25-2.33)	75 (50-101)	-	-
33.0*	1.50 ± 0.17	73 ± 6	40 ± 11	0.53 ± 0.12

* - Courses associated with central nervous system toxicity.
* - mean ± standard error.

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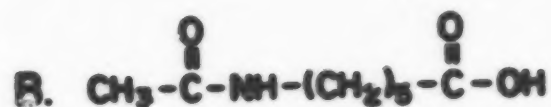
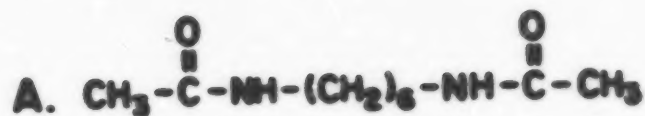
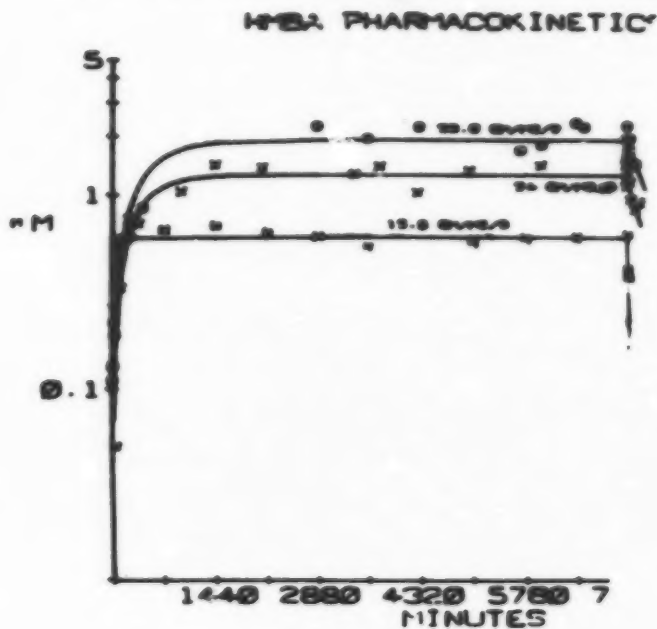


TABLE 2. PHARMACOKINETIC PARAMETERS

PATIENT NO.	DOSE (G/KG)	HALF-LIFE (MIN)	ELIMINATION CONSTANT (MIN ⁻¹)	AUC (MG-HR)	VOLUME OF DISTRIBUTION (L/KG)	SYSTEMIC CLEARANCE (ML/MIN/KG)
1	9.6	178	0.0009	2885	38.2	117
2	15.8	68	0.0115	4882	7.2	98
3	15.8	8	0.0851	4288	1.1	91
4	15.8	217	0.0002	17488	18.8	37
5	24.8	248	0.0008	1173	23.4	65
6	24.8	74	0.0125	4749	15.4	121
7	38.6	381	0.0003	13872	26.3	57
8	38.6	165	0.0042	9728	28.4	37
9	38.6	187	0.0035	7816	29.8	76

FIGURE 1. STRUCTURES OF HEXAMETHYLENE BISACETAMIDE (HMBA) (A), AND THE PRIMARY METABOLITE 6-ACETAMIDOHENANOIC ACID (B).



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TABLE 3. HMBA (x 10 DAYS) PATIENT CHARACTERISTICS

Number of Patients	12	
Number of Courses	17	
Sex Ratio (M:F)	6:6	
Median Age (Range)	61 (47-69)	
Performance Status	0 - 4	
	1 - 2	
	2 - 5	
	3 - 1	
Prior Therapy	Chemotherapy	4
	Radiotherapy	2
	Both	6
	None	0
Tumor Types	Colon	3
	Lung	3
	Sarcoma	
	(small intestine)	1
	Ovary	
	(mesothelioma)	1
	Lymphoma	1
	Biliary Duct	1
	Endometrial	1
	Breast	1

TABLE 4. HMBA (x 10 DAYS) DOSE ESCALATIONS

Daily Dose x 10 (gm/m ²)	No. of Patients	No. of Courses
12.0 ^a	1	1
15.8 ^a	7	10
20.0	5	6

^a One patient treated at both dose levels.

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TABLE 5. HMBA ($\pm$ 10 DAYS) TOXICITY

Dose (g/M ² /d)	No. of Courses	Toxicity Grade	Nausea and Vomiting	Thrombo- cytopenia	Leuko- penia	Skin	Renal	Acidemia/ Acidosis ^a
12	1 ^b	0	1	0	0	1	1	0/1
		1	0	0	0	0	0	
		2	0	0	1	0	0	
		3	0	1	0	0	0	
15	10	0	9	6	8	8	7	3/10
		1	0	1	2	1	3	
		2	1	2	0	1	0	
		3	0	0	0	0	0	
20	6	4	0	1	0	0	0	1/6
		0	4	0	1	6	4	
		1	1	1	3	0	1	
		2	1	1	1	0	1	
		3	0	1	0	0	0	
		4	0	3	1	0	0	

^a No. of courses with serum bicarbonate $\leq$ 20 meq/L.

^b One patient who developed grade 4 thrombocytopenia at 15.8 g/M²/d was retreated at 12 g/M²/d.

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HMBA
T84-0447
Johns Hopkins
November 1986

TABLE 6. EFFECT OF HMBA ON PLATELET COUNT

Dose (g/M ² /d)	Pretreatment Platelet Count ^a	Nadir Platelet Count (day)	Nadir/Pretreatment x 100 %
12	110	46 (d10)	42
15.8	130	24 (d11)	18
15.8	224	63 (d18)	28
15.8	450	207 (d 9)	46
15.8	375	141 (d11)	38
15.8	368	137 (d15)	37
15.8	560	214 (d15)	38
15.8	576	232 (d15)	40
15.8	960	590 (d15)	58
15.8	487	53 (d15)	11
15.8	440	90 (d15)	26
20	428	119 (d15)	28
20	239	63 (d15)	26
20	108	32 (d15)	29
20	320	19 (d18)	6
20	260	13 (d15)	5
20	332	21 (d18)	6

TABLE 7. HMBA (x 10 DAYS) SUMMARY OF EFFECTS

Hematologic	• Thrombocytopenia - nadir around d15-18, resolves - prior chemoRx and RT are r • Leukopenia (2/10 at 15.8 g/M², 5/6 at 20 g/M²) • Anemia
Renal/Metabolic	• ↑ BUN, Cr during infusion, resolves quick[*] (3/10 at 15.8 g/M²/d, 2/6 at 20 g/M²/d) • Acidemia/Acidosis (3/10 at 15.8 g/M²/d, 1/6 at 20 g/M²) • SIADH (1)
Neurologic	• No clinical CNS toxicity • No changes in Minimental exams • 6/14 post-treatment EEGs - mild diffuse cerebral function
Skin/Allergic	• Vasculitis
Other	• Nausea and Vomiting (4) • Hepatitis (Grade 1) (2) • Mucositis (1) • Malaise (1) • Anorexia (1) • Leukocytosis (7)

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TABLE 8. HMBA (x 10 DAYS) STEADY-STATE CONCENTRATION

Dose (gms/M2/d)	Mean Steady-State HMBA Concentration (range) (mM)	Mean Systemic Clearance (range) (cc/min/M2)
15.8*	0.63 (0.48-0.75)	89 (73-114)
15.8	0.35 (0.26-0.48)	164 (114-121)
15.8	0.23 (0.15-0.27)	46 (203-274)
15.8	0.45 (0.33-0.73)	126 (75-166)
15.8	0.48 (0.36-0.43)	140 (128-152)
15.8	0.77 (0.64-0.92)	72 (60- 85)
15.8	0.96 (0.59-2.10)	67 (26-107)
15.8*	1.68 (0.99-1.98)	35 (21- 55)
15.8*	1.26 (0.79-2.70)	50 (20- 69)
15.8	0.66 (0.26-1.28)	107 (43-211)
15.8 (mean $\pm$ s.e)	0.75 $\pm$ 0.14	110 $\pm$ 20
20.0	0.66 (0.55-0.75)	107 (93-126)
20.0	0.31 (0.25-0.36)	229 (193-257)
20.0	0.66 (0.53-0.84)	107 (89-131)
20.0	1.11 (0.79-1.70)	68 (41- 88)
20.0*	1.50 (0.95-3.40)	68 (20-182)
20.0*	1.72 (1.08-2.28)	43 (30- 64)
20.0(mean $\pm$ s.e)	0.99 $\pm$ 0.22	101 $\pm$ 27

* Developed ECOG grade 1-2 renal toxicity during course.

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INTRODUCTION

Carboplatin: (CBDCA, NSC241 240) is a second generation derivative of cisplatin, IN clinical testing it has been shown to be less nephrotoxic than the parent compound. The dose limiting toxicity, myelosuppression, makes it attractive for dose escalation in the autologous bone marrow transplant setting.

OBJECTIVES

- 1) To evaluate the pharmacology, toxicology, and other biomedical effects of CBDCA when myelosuppression is ameliorated by autologous bone marrow transplantation (ABMT).
- 2) To determine the efficacy of high dose CBDCA against refractory malignancy.

In the following tables, I will outline the interim results obtained for 15 patients treated with a total of 21 courses of CBDCA in dosages up to 1600 mg/m². A summary of this data will be provided at the conclusion of this presentation.

STMP

PATIENT ELIGIBILITY

Histologically documented malignancy,
Pathology reviewed at BMH or BIH
Stage 4 or locally unresectable,
Failed or ineligible or lack of any
curative or first line standard
standard palliative regimen
Aged 18-physiologic 55 years
Performance 0-1,
Prior treatment
3 weeks since RT or CT
1 week since surgery,
No prior pelvic irradiation*

REQUIRED LABORATORY DATA

White cell count >3000/ul.
Platelet count >100,000/ul.
Bilirubin, SGOT <1.5 x nl
Creatinine, BUN <1.5 x nl
GFR scan or
Creat. Clearance >60ml/m
FVC & DLCO* >70% predicted
BM Asp & Bx* No metastases
Brain CAT* No current metastases

*Only if bone marrow transplant planned

No previous or concomitant malignancy
No serious medical or psychiatric illness
No concurrent medications which interact with CBDCA metabolism.

SCHEMA: REGISTER

CBDCA mg/m²/day by 24 hour continuous infusion x 4 days.

Dose escalations:	/day	/course
	400	1600
	500	2000
	600	2400

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CBDCA
T85-0134
DFCI
November 1986

REGISTRY

upn:	age:	dx:	no. prev.	chemo:	CDDP:	response:
1	36	SCLC	1		Y	P.D.
2	57	SARCOMA	1		N	P.D.
3	34	CERVICAL	1		Y	P.D.
4	44	CERVICAL	0		Y	P.D.
5	38	OVARIAN	1		Y	P.D.
6	34	SARCOMA	3		Y	P.D.
7	64	OVARIAN	4		Y	C.R.
8	49	OVARIAN	1		Y	P.D.
9	63	OVARIAN	1		Y	C.R.
10	42	LUNG-ADEN	0		N	E.D.
11	24	TESTIC	4		Y	S.D.
12	51	OVARIAN	1		Y	P.R.
13	57	SCLC	1		N	
14	46	OVARIAN	1		Y	
15	41	OVARIAN	2		Y	

MYELOSUPPRESSION

upn	course #:	dose(mg/m2/4d):	days PMN<500:	days plate<50K:
2	1	375	0	0
7	2	400	1	12
1	1	500	0	0
5	1	600	4	18
4	2	600	0	5
6	2	750	0	0
3	1	800	10	12
4	1	900	4	7
6	1	900	6	11
9	2	900	10	7
7	1	1200	13	15
8	1	1200	17	18
9	1	1200	16	14
10	1	1200	-2	-2
11	1	1200	11	11
11	2	1200	9	5
11	3	1200	-2	-2
12	1	1600	13	8
13	1	1600	-2	-2
14	1	1600	-2	-2
15	1	1600	-2	-2

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MYELOSUPPRESSION BY DOSE

dose: (mg/m ² /4d)	days PMN<500:	Range:	days PLTS<50K:	Range:
900	5.33	6-11 days	8.33	7-11 days
1200	14	11-17days	14	11-18days
1600	13	13 days	8	8 days

NON HEMATOLOGIC TOXICITY

dose: (mg/m ² /4d)	creat. cl:		sgot:		bilirubin:		alk.	phos:
	int.	min.	int.	max.	int.	max.	int.	max.
900	52	52	21	21	1	1	94	94
900	69	69	23	23	.4	.7	110	146
900	77	-2	21	28	.4	.3	113	137
1200	55	54	24	24	.5	2.1	53	53
1200	91	-2	14	42	.5	15.3	92	313
1200	70	70	15	29	.6	.8	124	127
1200	143	-2	30	30	.3	1.5	104	256
1200	122	115	24	27	.7	1.2	92	138
1200	115	116	34	34	.3	1	96	96
1200	116	-2	46	-2	.7	-2	83	-2
1600	65	65	22	61	.5	2	71	366
1600	81	-2	28	-2	.7	-2	95	-2
1600	108	-2	31	-2	.4	-2	148	-2
1600	83	-2	19	-2	.8	-2	153	-2
X	88.92	77.29	26	30.78	.562	1.2*	102.77	157
S.D.	28.69	27	7.97	11.99	.21	.595	27.92	96.02

* significant at .02

Grade 3 Mucositis- 63 year old female with ovarian cancer

treated at 1200 mg/m². Culture Negative mucositis resolved as

WBC returned. No difficulty with second course of Rx at

900 mg/m².

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Elevated Bilirubin- heavily pretreated 49 year old female with ovarian cancer treated at 1200 mg/m². Patient was neutropenic with S.B.O., fevers and hematemesis at the time of maximum bilirubin of 15.2. There was no associated elevation of transaminase of alkaline phosphatase values and no evidence for hemolysis. Bilirubin value was 2.1 at time of discharge 21 days later.

Pulmonary Insufficiency- 42 year old female with NSCLC died while neutropenic of progressive respiratory distress. Autopsy results revealed extensive tumor involvement as the principle cause of death, but pulmonary toxicity from CEDCA could not be ruled out.

Hearing Loss- Patient with elevated bilirubin had profound loss of high frequency hearing. She had received extensive prior CDDP treatment and had experienced a transient loss of hearing at that time.

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Conclusions

- 1) 15 Patients have received a total of 21 courses of CBDCA in doses ranging up to 1600 mg/mt².
- 2) The principle toxicity has been dose related myelosuppression with PMNs <500 for 14 days and platelets <50,000 for 14 days in 4 evaluable patients treated at 1200 mg/mt².
- 3) Non-myelosuppressive toxicities have occurred sporadically.
 - a) one episode of grade 3 mucositis
 - b) one episode of grade 3 hearing loss
 - c) one episode of severe hyperbilirubinemia
 - d) one possible episode of pulmonary insufficiency
- 4) A small, but significant elevation in serum bilirubin has been observed in the majority of patients. This has not been associated with significant elevations in transaminase or alkaline phosphatase values.
- 5) Autologous bone marrow transplantation will probably be required at a dose of 2000 mg/mt².
- 6) The dose limiting toxicity for this study has not yet been reached.
- 7) Three of six evaluable patients treated at 1200 mg/mt² or 1600 mg/mt² have responded. These patients have all had previous combination chemotherapy.

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PHASE I-II TRIAL WITH 5-AZA-2'-DEOXYAZACYTIDINE (DAC) IN ADULT PATIENTS WITH ACUTE LEUKEMIA. P. Dodion (Institut Jules Bordet, Brussels, Belgium).

DAC is an analogue of azacytidine (fig 1). In vitro, it has cytotoxic properties; cytotoxicity increases with increasing DAC concentrations and increasing exposure time. Maximal cytotoxicity is reached for a concentration of 0.5 μ M and an exposure of 24 hr. In addition to its cytotoxic effect, DAC has differentiating properties. This differentiating effect is obtained at concentrations of 0.5-1 μ M and may be related to the inhibition of the DNA methyltransferase and the synthesis of undermethylated DNA.

In vivo, DAC is active against the L1210 leukemia; a better effect is obtained with repeated treatment or continuous infusion. DAC is also active against the intracerebrally implanted L1210 leukemia; a better effect is obtained with DAC than with cytosine arabinoside. Toxic effects of DAC in animals consist mainly of hematological toxicity.

The New Drug Development Coordinating Committee of the EORTC has initiated a comprehensive program of development of DAC; the program includes additional toxicologic studies, formulation, phase I and II trials in patients with solid tumors, and phase I-II trials in patients with leukemia. The present report focuses on the trial in leukemia.

DAC has been investigated in childhood acute leukemia by Rivard et al. The drug was given by continuous infusion for periods of 12-80 hrs. At doses $\leq$ 30 mg/kg over $\leq$ 30 hrs, no or minimal antileukemic activity was detected. In contrast, at doses of 40-80 mg/kg for 36-44 hrs, 2 complete remissions were achieved out of 9 patients. In addition, 2 patients with meningeal leukemia were improved. The drug was well tolerated.

On these grounds, a phase I-II trial was initiated in adult acute leukemia. This ongoing trial is conducted at the Institut Jules Bordet (Brussels, Belgium) and at the Hotel Dieu (Paris, France). The starting dose was 300 mg/sq m/d for 2 days (corresponding to approximately one half of the active dose in the Canadian trial). In the absence of excessive toxicity, the infusion duration was to be increased by steps of 24 hrs up to 120 hrs. This design was adopted to increase the exposure time while maintaining the drug concentration constant.

The patients' characteristics are listed in tables 1 and 2. The infusion time has been increased from 48 to 120 hrs (table 3). The protocol allowed patient's retreatment if eligibility criteria were still satisfied.

Non hematologic toxicity was generally mild to moderate (tables 4 and 5). Vomiting never required antiemetics. Liver function test abnormalities were observed in some patients but were not necessarily related to DAC therapy (table 6). Myelosuppression was the major problem in this study (tables 7 and 8). Myelosuppression was prolonged; several patients did not recover normal polymorphonuclear and platelet counts. However, all of them had reasons additional to therapy with DAC that could explain the absence of recovery (retreatment, infiltrated bone marrow, prior

autologous bone marrow transplantation) (table 9).

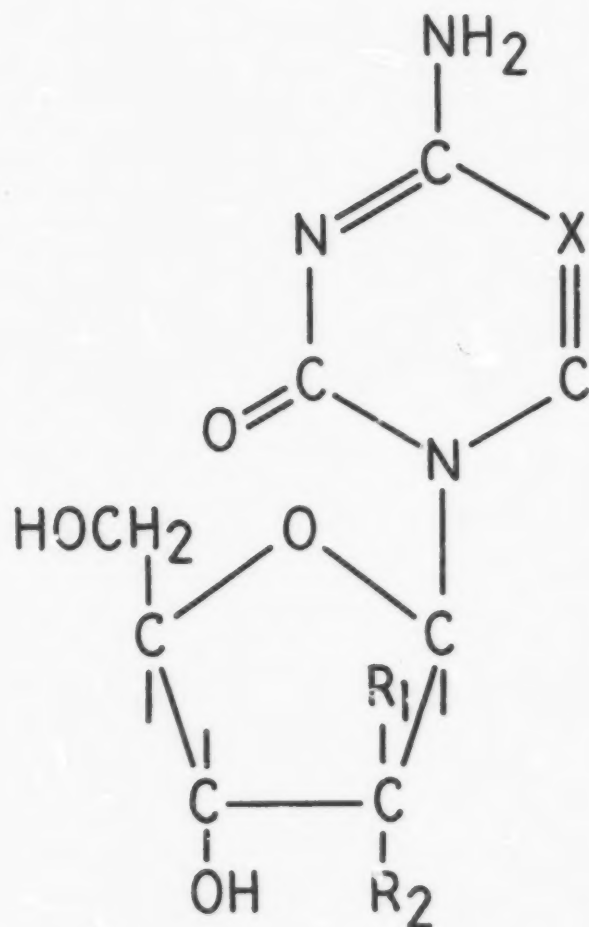
Antileukemic activity was detected in several patients; no complete response was achieved. However, partial remissions defined as a $\geq 75\%$ reduction in bone marrow blasts were obtained in 5 patients (table 10).

Pharmacokinetic analyses were performed using a bioassay based on the inhibition of the growth of L1210 cells. Plasma concentrations of DAC ranged between 0 and 0.05 μM during the infusion. These concentrations are substantially lower than those described by the Canadian investigators ($\pm 1 \mu\text{M}$) and by Pinedo et al in the phase I trial in patients with solid tumors (0.5-4 μM).

Subtherapeutic concentrations could be responsible for sub-optimal antileukemic activity. Residual leukemic cells could be responsible for the absence of hematologic recovery. Therefore, the treatment schedule of our trial has been modified in order to administer higher daily doses for a shorter period of time.

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STRUCTURES OF CYTIDINE, CYTOSINE ARABINOSIDE,
5-AZACYTIDINE AND 5-AZA-2'-DEOXYCYTIDINE.



compound	X	R ₁	R ₂
Cytidine	C	H	OH
Cytosine arabinoside	C	OH	H
5-azacytidine	N	H	OH
5-aza-2'-deoxycytidine	N	H	H

PHASE I TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE I: PATIENT'S CHARACTERISTICS (N=13)

Median age (range)	54 (24-73)
Median PS (ECOG) (range)	1 (0-3)
Male/female	8/5
Prior chemotherapy	13
Prior anthracycline antibiotics	9
Cytosine arabinoside	10
m-AMSA	5
5-azacytidine	1
Prior bone marrow transplantation	3
Allogenic	2
Autologous	1

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PHASE I-II TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE II: PATIENTS CHARACTERISTICS

LEUKEMIA TYPE	N PATIENTS	PHASE OF DISEASE				PREVIOUS CHEMOTHERAPY				PREVIOUS BONE MARROW TRANSPL.
		PHASE 1	REL 1	REL 2	REL 3	ARAC-CD	ARAC-HD	5-AZA	OTHER	
LYMPHOBLASTIC	1	-	-	1(1)	-	1	1	-	1	1
BIPHENOTYPIC	1	-	-	-	1(1)	1	1	-	1	-
NON LYMPHOBL.	8	1(1)	2(1)	4(2)	1(1)	7	5	5	8	2
CHRONIC MYELOID NON LYMPHOBL. CRISIS	3	3(1)	-	-	-	-	-	-	3	-

ARAC-CD : CONVENTIONAL DOSE ARAC; ARAC-HD : $>12 \text{ gr/m}^2/\text{COURSE}$; 5-AZA : 5-AZACYTIDINE
() IF AT LEAST ONE UNSUCCESSFUL TREATMENT FOR THE PHASE IN WHICH DAC WAS ADMINISTRATED

PHASE I-II TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE III: ENTRIES PER DOSE LEVEL

DAILY DOSE (MG/M ²)	TREATMENT DURATION (DAYS)	TOTAL DOSE (MG/M ²)	N° OF PATIENTS	INITIAL	N° OF COURSES (A) SUBSEQUENT	TOTAL
300	2	600	3	3	0	3
300	3	900	3	1	4	5
300	4	1,200	7	6	2	8
300	5	1,500	5	3	2	5
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(A) IN CASE OF RETREATMENT OF A PATIENT AT ANOTHER DOSE LEVEL, THE COURSE IS REPORTED AS SUBSEQUENT FOR THAT DOSE LEVEL.

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TABLE IV : NON HEMATOLOGICAL TOXICITY

TOTAL DOSE (MG/M ²)	N° OF COURSES	NAUSEA/ VOMITING	MUCOSITIS	LIVER TESTS ABNORMALITIES*	OTHER
600	3	2	0	0	1
900	5	5	3	1	2
1,200	8	4	4	4	2 (1)
1,500	5	3	0	1/2	2

() : N° OF COURSES WITH GRADE III-IV TOXICITY (WHO CRITERIA)

* : REPORTED ONLY IF INCREASE IN WHO GRADE POSSIBLY RELATED TO DAC

TABLE 5

OTHER NON HEMATOLOGICAL TOXICITIES

DESCRIPTION	TOTAL DOSE (MG/M ²)	DAY OF APPEARANCE	OUTCOME	RELATION WITH DAC
CARDIAC ARRHYTHMIA GRADE 1	600	2	SPONTANEOUS RECOVERY	POSSIBLE
MONOARTHRITIS	900	4	SPONTANEOUS RECOVERY	POSSIBLE
FEVER, GRADE 2	900	1	SPONTANEOUS RECOVERY	PROBABLE
HYDROSALINE RETENTION	1.200	1	IMPROVEMENT WITH DIURETICS	POSSIBLE
ACUTE RESPIRATORY DISTRESS	1.200	8	FATAL	POSSIBLE
REACTIVATION OF A PREVIOUS VENO- OCCLUSIVE DISEASE	1.500	?	?	POSSIBLE
CARDIAC ARRHYTHMIA GRADE 2	1.500	1 AND 8	REVERSIBLE WITH DIGITALIS	POSSIBLE

PHASE I TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE 6: LIVER FUNCTION TESTS ABNORMALITIES

Total dose mg/m ²	N° of patients	N° of courses	SGOT	SGPT	GGT	Alk. phosph.	Bili.
600	3	3	-	-	-	-	-
900	3	5	-	-	-	1	-
1200	7	8	-	1	2	2	2
1500	5	5	1	-	-	-	-

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PHASE I TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE 6B: LIVER FUNCTION TESTS ABNORMALITIES

Patient	Dose mg/m2	Abnormalities	Relationship with DAC
DU GI	300 x 3	.isolated increase of alk phosph max at d22, partially reversible, not observed at rechallenge	Unlikely
DR JE	300 x 4	.increase of bilirubin (2.2; post transfusion)	Unlikely
		.increase of alk phosph, max d8, partially reversible	Possible
		.increase of GT, max d8, partially reversible	Possible
FA KY	300 x 4	.increase of bilirubin, abnormal at start	Unlikely
ST XX	300 x 5	.increase of SGOT, transient	Possible

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PHASE I TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE 7 : NEUTROPENIA AND INFECTIONS (a,b)

Total dose mg/m ²	N° of courses		median (range) N° of days with Infection		
	eval. with PMN>500 at start		PMN<0.5	PMN<0.1	
600	2	2	4 (-)	0	0
900	5	3	29 (9-53)	4 (4-42+)	2
1200	8	7	37 (2+-83+)	7 (2+-53+)	5
1500	4	3	15+ (0-122)	10+ (0-17+)	3

(a) major infection (requiring broad spectrum antibiotics)

(b) duration of neutropenia: a + indicates no full recovery, generally because of death.

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PHASE I TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE 6 : THROMBOCYTOPENIA AND BLEEDING (a,b)

Total dose mg/m2	N° of courses		median (range) N° of days with platelets		Median N° of transfusions	Bleeding
	Eval.	With normal platelets at start	<50,000/ μ l	<20,000/ μ l		
600	3	2	7 (5+-15+)	9 (-)	1 (0-3)	0
900	5	4	42 (9-49)	19 (8-42+)	2 (0-4)	1 (+)
1200	8	3	55 (10+-95+)	35 (9-91)	7 (0-12)	2 (1+)
1500	5	0	16 (0-162+)	16 (0-162+)	6 (0-12)	3 (3+)

(a) only major bleeding episodes are listed into this table; (+) indicates a fatal bleeding.

(b) duration of thrombocytopenia: a + indicates no full recovery, generally because of death.

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PHASE I TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE 9 : CHARACTERISTICS OF PATIENTS WITH NO HEMATOLOGIC RECOVERY

Absence of recovery of neutrophils (n=7)

- death with infiltrated bone marrow	4
- prolonged hypoplasia, infiltrated bone marrow	1
- early death (acute respiratory insufficiency)	1
- death in aplasia (prior autoBMT)	1

Absence of recovery of platelets (n=8)

- death with infiltrated bone marrow	4
- retreatment with infiltrated bone marrow	1
- prolonged hypoplasia, infiltrated bone marrow	1
- early death (acute respiratory insufficiency)	1
- death in aplasia (11,000 platelets at start of DAC)	1

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PHASE I-II TRIAL WITH 2'-DEOXY-5-AZACYTIDINE IN ADULT ACUTE LEUKEMIA

TABLE 10: THERAPEUTIC RESULTS

TOTAL DOSE (MG/M ²)	N° OF COURSES	EVALUABLE COURSES	PROGRESSION	MINOR RESPONSE	MAJOR RESPONSE	COMPLETE REMISSION
600	3	3	1	2	-	-
900	5	5	-	3	2	-
1,200	8	8	-	5	3	-
1,500	5	2	2	-	-	-
TOTAL	21	18	3	10	5	0

PROGRESSION : INCREASE OF PERIPHERAL AND/OR BONE MARROW BLASTOSIS

MINOR RESPONSE : REDUCTION OF ≥ 75 % OF PERIPHERAL OR BONE MARROW BLASTOSIS

MAJOR RESPONSE : REDUCTION OF ≥ 75 % OF PERIPHERAL AND BONE MARROW BLASTOSIS LASTING FOR ≥ 1 MONTH

COMPLETE REMISSION : ACCORDING TO ALGB CRITERIA

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